CONSERVATION BIOLOGY OF TWO RATTLESNAKES, CROTALUS WILLARDI ODSCURUS AND SISTRURUS CATENATUS EDWARDSII

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CONSERVATION BIOLOGY OF TWO RATTLESNAKES, *CROTALUS WILLARDI OBSCURUS* AND *SISTRURUS CATENATUS EDWARDSII*

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By

ANDREW T. HOLYCROSS
MICHAEL E. DOUGLAS
ARIZONA STATE UNIVERSITY

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ABSTRACT

Anthropogenically exacerbated extinction rates are orders of magnitude above baseline and are rising rapidly, approaching and perhaps eclipsing those associated with the "terminal Cretaceous event." In western North America many species with narrow ecological tolerances have experienced natural declines and reductions in range as a consequence of a rapid warming and drying trend since the last pluvial period. Where these species' habitat requirements and human interests conflict, population declines, extirpations, and extinction often result. At the species level, conservation science attempts to answer questions associated with the biology of these declines and to expose connections between imperiled species and their environment.

Elucidating patterns of neutral genetic variation among and within populations of imperiled species can facilitate delineation of populations and reveal structure within populations, as well as offer insights to recent evolutionary history and its relationship to landscape. Insights gained from these studies are especially useful when considering questions of translocation and can also be valuable in legislative and legal battles. Equally important to conservation of species, though often underappreciated, is an understanding of species' autecology. In many cases, basic natural and life history data have not been gathered.

In this research, patterns of variation at neutral DNA markers are explored in order to characterize relationships among and within populations of two endangered rattlesnakes [*Crotalus willardi obscurus* (New Mexico Ridgenose Rattlesnake) and *Sistrurus catenatus edwardsii* (Desert Massasauga)]. These data demonstrate that

geographically discrete populations of both species are also genetically isolated and on independent evolutionary trajectories. These data also offer insights to the demographic history and genetic health of assayed populations. Within the context of these findings, conservation implications of natural and life history data (foraging ecology and reproduction) gathered over the course of two, five-year field studies are considered, as well as a specific threat to the woodland habitat of *Crotalus willardi obscurus*.

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Introduction

Exposing and preserving biodiversity is the primary goal of contemporary conservation biology. Recently, this objective has been most often pursued using molecular markers that illuminate patterns of diversity and corresponding evolutionary history. Though such approaches are important, if not critical, to managing imperiled taxa, they offer few insights to the ecological needs of the populations they delineate. Therefore, site-specific natural history studies that determine the conservation needs of populations in the context of their biotic communities are essential. Although they are often under-valued by contemporary biologists, descriptive natural history studies have been, and will continue to be, instrumental in both conservation of populations (Dodd, 1987; Greene, 1994) and development of biological theory (Darwin, 1859, 1906). Another benefit of studying local populations of widespread but endangered organisms is that in the absence of molecularly based inferences, geographic patterns of variation in natural history characters can often provide reasonable insights to possible phylogeographic scenarios, insights that can later be tested using appropriate molecular methods. Patterns of variability in natural history characters may or may not be concordant with evolutionary relationships among populations as revealed by molecular methods. Regardless of concordance, evaluating patterns of variability in natural and life history characters relative to recovered phylogeny allows a broader understanding of evolutionary history (Greene, 1994) and possible co-evolution of natural history character types.

Constrained as limbless, gape-limited predators, snakes require special consideration in studies of natural history variation and in identifying conservation

research priorities. Dietary and foraging studies are particularly important for understanding snake biology, as diet is a primary and limiting force in evolution of snake morphology (Arnold, 1993; Forsman and Shine, 1997), physiology (Cock Buning, 1983; Secor and Diamond, 1998), community structure (Reynolds and Scott, 1982; Vitt, 1983; Rodríguez-Robles and Greene, 1996), movement/activity patterns (Duvall et al., 1990b; Secor, 1995; Madsen and Shine, 1996) and habitat use (Reinert et al., 1984; Chandler and Tolson, 1990). Many snakes are relatively specialized predators with geographically and/or ontogenetically variable diets and thus may be particularly vulnerable to ecological changes that affect foraging strategies or prey populations. Dietary assays also provide insights into the evolution of natural and life history characters (Shine, 1996; Rodríguez-Robles et al., 1999); insights that may have significant implications for conservation of populations (e.g., Downes and Shine, 1998; Shine et al., 1998). As landscapes become increasingly fragmented and climate change alters species distributions (Meffe and Carroll, 1997), a thorough knowledge of trophic ecology is of paramount importance in snake conservation. Unfortunately, information regarding the diet of many snakes is anecdotal at best (Mushinsky, 1987), due in part to the difficulty of obtaining large sample sizes.

Assessing the viability of small, isolated, and sexually reproducing populations also requires descriptive studies of reproduction. However, key reproductive and other life history characters vary nearly as much within species as among them, probably due to rapid response of life history traits to geographically variable selection pressures (Reiserer, 2001). Thus, studies specific to populations of concern, or studies that assess geographic variation in these variables are preferable for conservation applications. As

studies of reproduction accumulate across a range of snake taxa, they will be of significant aid in efforts to untangle the evolution of ophidian life history strategies (Reiserer, 2001). Done well, such studies require large sample sizes and intensive field study that often prove logistically or economically problematic. Thus, regrettably, for most snake populations, data on reproduction are insufficient for even gross estimates of natality, a factor critical to assessing a population's biotic potential (Parker and Plummer, 1987; Seigel and Ford, 1987).

If the aim of conservation is to preserve both biodiversity and evolutionary processes, populations are the appropriate unit of management. This is the level at which demographic and genetic processes are currently operating, and the level at which extinctions occur (Meffe and Carroll, 1994). Data from neutral nuclear DNA markers can expose patterns of variation that can be used to define local populations, elucidate population substructure, determine levels of inbreeding, and even assess recent demographic history (Avise, 1994; Garza and Williamson, 2001). With such information managers are better equipped to define management units (*sensu* Moritz, 1994), prioritize populations for conservation, and rank populations with regard to their conservation value.

Levels of genetic variability within isolated populations is largely a consequence of population size (extant and historical) and degree of isolation, but is also theoretically tied to future viability. Where population sizes are small (or have bottlenecked in the past) and/or levels of gene flow are negligible, populations are susceptible to loss of genetic variability via inbreeding or genetic drift and are theoretically at increased risk of extinction. The use of neutral, nuclear DNA markers, particularly microsatellites, for

assessing these patterns in a wide variety of taxa has exploded in recent years due to the relative efficacy of lab procedures. However, snakes have been underrepresented in such evaluations, due primarily to their secretive nature and the concomitant difficulty of acquiring suitable sample sizes for analysis. Relatively few studies have evaluated nuclear DNA variation in snakes (Gibbs et al., 1994; Gibbs et al., 1997; Prior et al., 1997; Bushar et al., 1998; Lougheed et al., 1999; Prosser et al., 1999).

Herein, I use microsatellite DNA loci to describe population structure and explore the implications of patterns of variability at these loci for conservation. The reproductive and foraging ecology of two threatened species is explored in four chapters. Additionally, a specific threat to *Crotalus willardi obscurus* is explored in a chapter on the effect of prescribed fire on mortality, behavior and most importantly, habitat. These studies provide some of the basic building blocks necessary for population viability analyses and formulation of recovery plans. A synopsis of the biogeography of the area and the biology of each focal species is provided for context.

Biogeographic History of the Madrean Archipelago and Focal Species

Post-middle Miocene block-faulting (the "Basin-Range disturbance"; Menges and Pearthree, 1989) and temporally associated minor volcanism created the characteristic range-valley physiography of the Mexican Highland geologic subprovince (Eberly and Stanley, 1978; Menges and Pearthree, 1989). Concomitant and subsequent sedimentation filled intervening basins to their present depths of up to 4,000 m (Scarborough and Pierce, 1978). Integration of most of these bolsons with external drainage systems resulted in dissection of basins during the Pliocene and Pleistocene (Scarborough and Pierce, 1978).

Increased winter precipitation, combined with cooler summer temperatures and mild winters during the last glacial maximum (~18 kyr BP), may have supported extensive Pleistocene pine and spruce forests in these intermontane valleys as evidenced by 1) palynological samples from Willcox Playa (pluvial Lake Cochise) and other lacustrine deposits (Martin, 1963a, 1963b; Martin and Mosimann, 1965), 2) fossil pollen in packrat (*Neotoma* spp.) middens (Anderson and Van Devender, 1995), and 3) macrofossils collected from packrat middens (Spaulding and Graumlich, 1986; Van Devender, 1995 [and citations therein]). Pollen spectra from late Pleistocene Lake Cochise occur in proportions comparable to those of contemporary spectra in extant pine/spruce parkland at Deadman Lake (2,600 m) in the Chuska Mountains of northwestern New Mexico (Martin, 1963a). Plant fossils and pollen found in packrat middens reveal a pinyon-juniper forest existed as low as 795 m in the Waterman Mountains near Tucson (Anderson and Van Devender, 1991).

Around 12 kyr BP decreases in winter rains and increases in summer temperatures promoted a general drying trend (Antevs, 1955; Spaulding and Graumlich, 1986) causing the gradual isolation of oak and pine woodlands to mountain refugia (Betancourt et al., 1990; Van Devender et al., 1987; Haynes, 1991). Increasing summer monsoon rains from ca. 9 kyr BP to 4 kyr BP (Martin, 1963; Spaulding and Graumlich, 1986) supported the spread of mesic desert grasslands (in comparison with contemporary desert grasslands) in valleys and on bajadas (Van Devender, 1995). Indeed, fossil vertebrates from Howell's Ridge cave (Little Hatchet Mountains, New Mexico) indicate that during the middle Holocene the Playas Valley supported a thriving grassland community as well as a perennial pluvial lake with fish (Colorado chub, *Gila robusta*) and microtine rodents (Van Devender and Worthington, 1977). Since 4 kyr BP, waning monsoons and periodic droughts have caused lake desiccation and promoted the invasion of grasslands by desert scrub species (Van Devender, 1995). Nevertheless, healthy grasslands dominated the San Simon, San Bernardino, and Sulphur Springs Valleys prior to 1880 (Bahre, 1995). Late nineteenth century drought combined with intensive grazing at the turn of the century (and continued grazing during the twentieth century) exacerbated grassland decay, resulting in the doubling of scrub-dominated lands in southern Arizona by 1952 (Parker and Martin, 1952). Many historically lush grasslands in the area (*e. g.*, San Bernardino Ranch, the flats northeast of Douglas, the San Simon Valley between San Simon Cienega and Portal) are now desert scrub rent by channelization and headward cutting of washes and streambeds (Bahre, 1995). The once contiguous desert grasslands of the San Bernardino and Sulphur Springs Valleys now persist as relict isolates.

It is often assumed that faunas associated with modern plant communities "tracked" these communities as they moved up and down elevation gradients during climatic fluctuations. This argument assumes that plant communities have remained largely unchanged, and simply moved as a whole (a concept with roots in Clementsian climax theory). However, it appears that not all components of a community responded to climatic fluctuations in the same manner. Palaeocommunities were often very different in composition from modern analogues. For example, many plant (and reptile) species characteristic of desert scrub today occupied low pinyon-juniper woodlands in the late Pleistocene (Van Devender and Mead, 1978; Van Devender et al., 1991). Yet other species, characteristic of contemporary montane habitats, appear to have been extirpated from intermontane Pleistocene communities that phytogeographically approximate modern habitat on adjacent mountains (Van Devender et al., 1991).

Today, the mountains of the Madrean Archipelago and their associated grassland valleys are reknowned as centers of species diversity and for their mosaic communities. Some of the less vagile members of the faunas associated with these communities have presumably been isolated on island refugia for ca. 4–11 kyr, and may be independent evolutionary entities. *Crotalus willardi* (Ridgenose Rattlesnake) typifies the distribution of many montane species in the isolated northern ranges of the Sierra Madre Occidental. Similarly, *Sistrurus catenatus edwardsii* (Desert Massasauga) is found in relict grasslands, presumably isolated by xerification and more recently desertification. The fragmented distribution of these local populations typifies the situation for each species throughout much of its range.

Fragmentation and isolation of these montane forest and desert grassland habitats has presumably led to the isolation and divergence of many terrestrial animals of low vagility. However, direct evidence of post-Pleistocene (Madrean forests) or late Holocene (desert grassland) isolation of vertebrates currently associated with these communities is lacking. If presumed barriers to migration and gene flow are not as effective as hypothesized, geographic units may not be significantly divergent. Alternatively, isolation may have been effected much earlier if 1) habitats were not contiguous in the past as suggested by the palynological and historical record or 2) not all faunal elements 'tracked' their communities as currently presumed. These alternative scenarios have differing implications for management of these island populations. Delineating evolutionarily significant units for conservation based on presumed phytogeographic history alone is not advisable.

Focal Species: Biogeography, History of Study, and Autecology

Crotalus willardi (Ridgenose Rattlesnake) is a small montane pitviper that is distributed throughout the Sierra Madre Occidental of Mexico with northern populations isolated on mountains in extreme southwestern New Mexico and southeastern Arizona. Barker (1992) described the infraspecific relationships of five subspecies based on limited variation in allozymes and morphology. Published life history and natural history data for this species are extremely limited, consisting primarily of anecdotal accounts of individuals encountered in the wild and notes from captive propagation.

Crotalus w. obscurus (New Mexico Ridgenose Rattlesnake), the most recently described subspecies (Harris and Simmons, 1976; Barker, 1992), occurs in the Sierra San

Luis (Mexico) and in the neighboring Animas and Peloncillo Mountains (United States). Different histories of isolation and rates of reduction in population size have likely contributed to different genetic circumstances for each population. Allozymic variation was found at three loci in the Sierra San Luis population. However, these loci were monomorphic in limited samples from the Animas and Peloncillo Mountains (Barker, 1992). Nevertheless, past or extant genetic exchange between the Sierra San Luis and Animas Mountains via high elevation passes that connect these two ranges with the Sierra San Luis is theoretically possible. The broad Animas Valley separates the Peloncillo and Animas Mountains and is probably a complete barrier to migration.

Crotalus w. obscurus was listed as 'threatened' under the Endangered Species Act on 04 August 1978 (USFWS, 1978). A Species Recovery Plan (Baltosser and Hubbard, 1985) recommended *in situ* study and establishment of a captive breeding program based on extremely limited information. Status of populations was difficult to determine, and was based on less than 100 encounters throughout the subspecies' range (Baltosser and Hubbard, 1985).

During 1988 and 1989 a preliminary study of the San Luis population was conducted allowing preliminary evaluations of movement, diet and habitat use (Barker, 1991). Based on elevation and size, the Sierra San Luis probably support the largest population. The Animas population was discovered in 1957 (Bogert and Degenhardt, 1961). Aside from an intense period of commercial collection during the 1970s, the Animas population has been relatively unaffected by humans (for a history of impacts on *C. w. obscurus*, see Applegarth, 1980). The New Mexico Department of Game and Fish Endangered Species Program began capture-recapture efforts (using PIT tags) on Animas

Mountain in 1991 (C. W. Painter, unpubl. data). Currently the Animas Mountains, in their entirety, are managed as a privately owned ranch and nature preserve and are closed to the public. The Peloncillo population, described from a hybrid *C. willardi* X *C. lepidus* (Campbell et al., 1989), occurs on the Coronado National Forest and has an ongoing history of exploitation by commercial collectors and reptile enthusiasts. The Peloncillo Mountains are much lower in elevation, with limited pine-oak habitat, and appear to support a small and low density population of *C. w. obscurus*. The recent use of prescribed fire to reduce woody vegetation and establish grasslands in much of the Animas and Peloncillo Mountains has the potential to severely affect habitat for *C. w. obscurus*. Preliminary mark-recapture and telemetry data from the Animas Mountains and Sierra San Luis are consistent with the notion that philopatric demes of rattlesnakes are centered in wooded canyons in these mountain ranges.

Sistrurus catenatus (Massasauga) ranges from New York state to southeastern Arizona (Stebbins, 1985; Conant and Collins, 1991). The species also occurs in both Mexico and Canada (where it is endangered) although its range in both of these countries is extremely limited or fragmented (Stebbins, 1985; Conant and Collins, 1991; Greene and Campbell, 1992; Gibbs et al., 1997). Three subspecies are recognized (based on poorly defined variation in pattern and meristic characters): *S. c. catenatus* (Eastern Massasauga), *S. c. tergeminus* (Western Massasauga), and *S. c. edwardsii* (Desert Massasauga; "Arizona's rarest snake", Lowe et al., 1986). *Sistrurus catenatus* is legally protected in every state in which it is found and *S. c. catenatus* is a candidate for listing under the Endangered Species Act. Preferred habitat in the east consists of lowland wet

meadows, swamps, bogs, cienegas, streams, and seasonally moist grasslands. Populations in the desert southwest occur in desert grasslands, shortgrass prairie, and dune formations (Ernst, 1992; Degenhardt et al., 1996). All three putative subspecies appear to have locally narrow ecological tolerances, as evidenced by decline and disappearance of populations across their geographic range. The primary threat to conservation is degradation, fragmentation, or destruction of habitat (Seigel, 1986; Dodd, 1987; Greene and Campbell, 1992), although road mortality and willful extermination may be regulating factors for some populations.

Southeastern Arizona populations of *S. c. edwardsii* are isolated island populations much reduced in geographic range due to desertification and fragmentation of a once continuous semi-desert grassland in the San Bernardino, San Simon, and Sulphur Springs Valleys (Humphrey, 1958; Hastings, 1959; Humphrey, 1987). The San Bernardino Valley supports the largest extant population of Massasauga in Arizona. In the Sulphur Springs Valley, failed agricultural attempts have drastically reduced suitable habitat, perhaps resulting in the extirpation of *S. c. edwardsi*. However, a single recent specimen suggests that the population may be extant, albeit at low density.

Virtually nothing is known of the natural and life history of *S. c. edwardsi*. While the autecology of *S. c. catenatus* has been extensively studied (e.g. Wright, 1941; Greene and Oliver, 1965; Keenlyne and Beer, 1973; Keenlyne, 1978; Reinert, 1981; Reinert and mm Kodrich, 1982; Seigel, 1986; Weatherhead and Prior, 1992), *S. c. edwardsii* is probably as different from its eastern relative in ecology as it is in morphology.

The San Bernardino Valley population occupies a tobosa (*Hilaria mutica*) grassland of ca. 150 square km and is known almost exclusively from road-collected

specimens (Lowe et al., 1986). Significant attrition from road mortality (ca. 40% of specimens collected between 1993 and 1995 were collected dead on the road) and collection by hobbyists (despite legal protection) appear to be major problems. Effects of current grazing on this population are arguable. Intense livestock grazing has been demonstrated to decrease abundance of desert grassland reptiles dependent on bunchgrass cover (Bock et al., 1990). While *S. c. edwardsii* populations rely on tobosa bunchgrass for cover, they do so during the late summer and early fall "monsoon season" (Holycross, unpubl. data.), when grazing has limited impact on tobosa cover. It has been suggested (H. W. Greene, pers. comm.) that mesquite removal ("grubbing") by local ranchers may be partially responsible for the persistence of this desert grassland and its fauna. Photographs (Bob Krentz) taken of the study site during the 1930's depict a landscape virtually devoid of vegetation, thus existing conditions may not represent historical conditions. However, this study is not an evaluation of the effects of grazing on *S. c. edwardsii* populations. Rather, it might offer insights to the effect historic grazing (and other factors) may have had on population structure and genetics via fragmentation of habitat.

Reproduction in the Desert Massasauga, *Sistrurus catenatus edwardsii*, in Arizona and Colorado.

Conservation of relict populations of long-lived, iteroparous species requires baseline information on reproduction and phenology. These data are especially important in assessing threats to small populations where high levels of genetic differentiation among neighboring populations suggest independent demography (e.g. Gibbs et al., 1997). Reproductive data can be gathered through histological examination of museum specimens when suitable sample sizes of archival material are available. However, specimens of rare and declining taxa are often lacking in museums and collecting specimens from wild populations may not be an ethical or legal option. Herein I present information on reproduction from histological examination of gonads from two disjunct populations (ca. 845 km apart) of *Sistrurus catenatus edwardsii* (Viperidae) from Arizona and Colorado. I also include radio-telemetric and mark-recapture observations from the Arizona population. The purpose of this chapter is to expand our knowledge of the reproductive biology of this rare, secretive, and protected snake using data collected almost exclusively from animals found dead on roads (DOR) or from live animals *in situ.*

Across its broad distribution in North America, *Sistrurus catenatus* persists in isolated populations. Climate change, habitat destruction, and regionally narrow habitat use all appear to have shaped this fragmented distribution (Reinert and Kodrich, 1982; Greene and Campbell, 1992). The reproductive biology of eastern and midwestern populations has been studied (summarized by Fitch, 1970; Minton, 1983; Seigel, 1986, and Ernst, 1992). However, there is little information on *S. c. edwardsii.* Reproductive phenology for Arizona *S. c. edwardsii* is known solely from captive animals (Lowe et al.,

1986), while no information appears to exist for this species from Colorado (Hammerson, 1986).

METHODS AND MATERIALS

Fifteen female and 20 male Arizona *S. c. edwardsii* from the collections of Arizona State University (ASU) and the University of Arizona (UAZ) were examined; also included are data from four neonates and 24 additional Arizona females (one of which was radio-tracked) that were captured and released. All Arizona snakes were from Cochise County and were collected in 1969, 1972, 1981 and 1993–1996. All ASU specimens were DOR snakes collected 1993–1996 as part of a long-term ecological study of *S. c. edwardsii* in desert grasslands. The Arizona study site is a high-elevation desert grassland situated on the divide between the San Simon and San Bernadino Valleys (31°42'N, 109°06'W, ca. 1370 m elevation). Tobosa (*Hilaria mutica*) dominates the site with other grasses occurring in lower densities.

Also examined were seven female, 17 male and three neonate Colorado *S. c. edwardsii* from the herpetology collection of the University of Northern Colorado, Greeley (UNC). All Colorado specimens were found DOR and were collected 1996–1997 from Baca, Cheyenne, Kiowa and Lincoln Counties. In Colorado, *S. catenatus* inhabits dry plains grasslands (Hammerson, 1986). Colorado populations of *S. catenatus* have been regarded as intergrades between *S. c. tergeminus* and *S. c. edwardsii* (Maslin, 1965; Stebbins, 1985; Conant and Collins, 1991), but a recent evaluation of geographic variation in morphological and ecological characters suggests that Colorado populations should be regarded as *S. c. edwardsii* (Hobert, 1997; S. P. Mackessy, pers. comm.).

The left gonad was removed (30 testes, 19 ovaries), embedded in paraffin, and cut into histological sections at 5 μm. Because all males were DOR snakes (except for UAZ 34695 from which testes were missing), not all parts of the reproductive tracts were available for study from each snake. Of 37 males, testes were available in 30, epididymides in 8, and vasa deferentia in 24. Kidney sexual segments from 15 of 17 Colorado and four of 20 Arizona males were examined for secretory granules; two from Colorado males were not available for study (one from May was autolyzed, one from June was damaged). All slides were stained with Harris' hematoxylin followed by eosin counterstain. Testis slides were examined to determine the stage of the male cycle; ovary slides were examined for the presence of yolk deposition.

Results and Discussion

From Arizona: females (N = 39) measured 380 mm (mean SVL) ± 36.5 SD, range = 329 to 523 mm; males (N = 20) measured 368 mm ± 60.1 SD, range = 298 to 541 mm; neonates (N = 4) measured 168 mm ± 5.9 SD, range = 162 to 176. From Colorado: females (N = 7) measured 358 mm ± 26.0 SD, range = 330 to 398 mm; males (N = 17) measured 374 mm ± 55.2 SD, range = 280 to 473 mm; neonates (N = 3) measured 198 mm ± 9.6 SD, range = 188 to 207. Testicular histology was similar to that reported in the viperid snake, *Agkistrodon piscivorous* by Johnson et al. (1982) and the colubrids, *Masticophis taeniatus* and *Pituophis catenifer* (= *melanoleucus*) by Goldberg and Parker (1975). In the regressed testes, seminiferous tubules contained spermatogonia and Sertoli cells. In recrudescence, there was renewal of spermatogenic cells characterized by spermatogonial divisions; primary and secondary spermatocytes and spermatids were

sometimes present. In spermiogenesis, metamorphosing spermatids and mature sperm were present.

Monthly distribution of stages in the testicular cycle (Arizona and Colorado samples combined, no obvious phenological differences between them) is shown in Table 1. The smallest reproductively active male (spermiogenesis in progress) measured 280 mm SVL. Males measuring less than 280 mm SVL were excluded from the study to avoid bias from including sub-adults in the analysis. This size is smaller than the minimal size for female reproductive activity (329 mm SVL) found in this study and might suggest males mature at an earlier age than females. Further investigations will be needed to answer this question.

Small sample size prevented a complete description of reproductive phenology, however I found males undergoing spermiogenesis in June through October. Histological examination of vasa deferentia revealed sperm present in males in the following proportions: 7/7 April, 3/3 May, 7/7 June, 2/2 August, 4/4 September, 1/1 October; examination of the epididymides revealed sperm present in the following proportions: 3/3 April, 1/1 May, 0/1 July, 1/2 August, 1/1 October. The testicular cycle of *S. c. edwardsii* appears to fit the aestival spermatogenesis category of Saint Girons, 1982 in which spermiogenesis ends in September or October. A variety of New and Old World snakes have testicular cycles fitting this pattern (Saint Girons, 1982; Seigel and Ford, 1987). Male kidney sexual segments were enlarged and contained secretory granules as follows: 7/7 April, 1/1 May, 1/1 June, 3/3 August,4/4 September and 3/3 October. Mating is known to coincide with hypertrophy of the kidney sexual segment (Saint Girons, 1982).

Ernst (1992) suggested that the breeding period for *S. catenatus* extended from March to November. Lowe et al. (1986) reported that *S. c. edwardsii* mates in both spring and fall in Arizona, based on observations of captive snakes. The presence of sperm in the vasa deferentia of *S. c. edwardsii* from April through October suggests males are capable of inseminating females throughout this period. Field observations of copulating *S. c. edwardsii* will be needed to ascertain when breeding occurs.

To avoid the possibility of including sub-adult females in analysis of the ovarian cycle, I excluded females below the minimum size of the smallest gravid female (329 mm SVL) found during the study. Only 15% (7/46) of the females were reproductively active (vitellogenic follicles or gravid; 3/7 Colorado, 4/39 Arizona). Proportions of reproductively active females from Arizona (combining data from museum specimens and captured and released females) were: May 0/2; June 2/2; July 1/2; August 1/18; September 0/8. For Colorado females using data from museum specimens, I had the following ratios: April 1/2; May 2/2; August 0/3. These observations indicate that only part of the *S. c. edwardsii* female population reproduces each year, as previously suggested for *S. c. catenatus* by Reinert (1981), who found 15/26 (58%) gravid adult females in Pennsylvania. In Missouri, the percentage of reproductive females varied from 33 to 71% over three years (Seigel, 1986). Seigel et al. (1998) reported a significantly lower percentage of gravid female *S. catenatus* from Missouri during 1993–1994 (23%), after a severe flood in 1993, as opposed to 1979–1083 (50%). These data, gathered over four (Arizona) and two (Colorado) field seasons, may suggest that females from these populations reproduce less frequently than other populations studied, although in view of

the findings of Seigel et al. (1998), one must consider that percentages of gravid females may vary markedly in different years.

Litter sizes from the Arizona sample were determined by palpating live females found in the field. Colorado litter sizes were determined through counting enlarged follicles of preserved specimens. Mean litter size for Arizona females (N = 4) was 5.8 ± 1.7 SD, range 4–8; for Colorado females (N = 2) 4.5, range = 4–5. Mean litter size of both populations pooled was 5.3 ± 1.5 SD, range = 4–8 (Table 2).

There is considerable variation in *S. catenatus* brood sizes in different parts of its range. Fitch (1970) summarized data on 54 *S. catenatus* litters (from Klauber, 1956) ranging from 2–19, and calculated an average litter size of 8.16 ± 0.44 SE. Fitch (1985) later, using different data, reported a range of 3–13 for 115 *S. catenatus* litters from much of its range in the United States; litter sizes appeared to increase from east to west and from south to north. Seigel (1986) reported a mean litter size of 6.4 (range = 4–10) for 17 *S. catenatus* from Missouri and summarized variation in litter size for *S. catenatus* in different parts of its range (range = 3–19). Keenlyne (1978) reported 58 *S. catenatus* from Wisconsin had 11.1 young per female. Lowe et al. (1986) reported values for two litters of five and seven, born to captive females from Arizona. While litter sizes of *S. c. edwardsii* in this study (range = 4–8) are within the ranges previously reported for this species, they tended towards the lower end of the published ranges for the species.

Phenology of parturition was inferred from first appearance of neonates and cessation of the appearance of gravid females during continuous mark-recapture

TABLE 1. Distribution of conditions in seasonal testicular cycle of *Sistrurus catenatus edwardsii* from Arizona and Colorado. Values are the number of males exhibiting each of the three conditions.

Month	n	Regressed	Recrudescence	Spermiogenesis
April	6	6	0	0
May	3	1	2	0
June	2	0	1	1
July	10	1	1	8
August	5	0	0	5
September	1	0	0	1
October	3	0	0	3

TABLE 2. Seasonal occurrence of reproductive females and litter sizes in *Sistrurus catenatus edwardsii* from Arizona (wild-caught) and Colorado (DOR).

State	Female SVL (mm)	Capture Date		Litter size
AZ	418	5	June	5 embryos
AZ	390	22	June	6 embryos
AZ	329	21	July	4 embryos
AZ	422	2	August	8 embryos
CO	362	24	April	4 follicles > 4 mm
CO	362	30	May	5 follicles > 13 mm
CO	382	17	May	damaged vitellogenic follicles

collecting efforts over four years (1993 to 1996), in addition to radio-telemetric observation. A gravid Arizona female collected 2 August 1995 was implanted with a radio transmitter and located daily until she gave birth on ca. 11 September 1995, when she was observed with a single, moist neonate. During the course of field studies in Arizona, four neonates were captured and released: (1) 166 mm SVL, 5.1 g, 6 September; (2) 162 mm SVL, 3.9 g, 10 September; (3) neonate with fresh umbilicus, 167 mm SVL, 4.9 g, 11 September; (4) 176 mm SVL, 3.4 g, 28 September. Three Colorado *S. c. edwardsii* were collected in early October: (1) 188 mm SVL, 5 October; (2) 207 mm SVL, 5 October; (3) 200 mm SVL, 10 October. These observations suggest that Arizona and Colorado *S. c. edwardsii* populations give birth from late August through September, perhaps later in the year when compared with species-wide reports of parturition from late July to late September (Ernst, 1992). All of the Arizona neonates weighed less than the range of neonatal weights reported for the species as a whole (8 to 10 g) by Ernst (1992).

Additional observations are required before definitive comparisons can be made between reproductive characteristics of eastern and western populations of *S. catenatus*. Unfortunately, due to the difficulty of collecting these secretive, rare, and protected snakes, it is unlikely that significant additions will be made to data sets in the near future.

Reproduction in Northern Populations of the Ridgenose Rattlesnake, *Crotalus willardi* (Serpentes: Viperidae).

Comprehensive knowledge of reproductive biology is of paramount importance to understanding the evolution of ophidian life history strategies (Seigel and Ford, 1987). Such data are also critically important to assessing conservation needs and threats to small, relict populations of imperiled species. For many such taxa, data on reproduction are insufficient for even gross estimates of natality, a factor critical to assessing a population's biotic potential. One such organism is the Ridgenose Rattlesnake, *Crotalus willardi*, a small montane species distributed from Zacatecas, México north into southeastern Arizona and southwestern New Mexico within the Sierra Madre Occidental and associated ranges (Campbell and Lamar, 1989). Five subspecies are recognized: *C. w. amabilis*, *C. w. meridionalis*, *C. w. silus*, *C. w. obscurus* and *C. w. willardi* (Barker, 1992). *Crotalus w. obscurus,* and *C. w. willardi* consist wholly of isolated populations occupying northern outliers of the Sierra Madre Occidental (Greene, 1997) known as the "sky islands" or "Madrean Archipelago". *Crotalus w. obscurus* is listed as "threatened" under the Endangered Species Act (U.S. Fish and Wildlife Service, 1978) and populations of *C. w. willardi* are protected by the state of Arizona. Although many of these isolated populations appear sufficiently large that stochastic events are not a threat to persistence, capture rates in some populations occupying marginal habitat (e.g. *C. w. obscurus* in the Peloncillo Mountains and *C. w. willardi* in the Whetstone Mountains) suggest dangerously small populations (ATH, unpubl. data; D. Turner, pers. comm.). Many aspects of the natural and life history of this species are becoming better understood through intensive field studies (Smith et al., 2001; Holycross et al., *In press*).

Nevertheless there are only anecdotal accounts of reproduction in *C. willardi* and several of these are based on captive snakes (Armstrong and Murphy, 1979, Delgadillo Espinosa et al., 1999; Holycross, 2000 and citations therein). Here, I present a synthesis of information on the reproductive biology of *C. willardi* based on long-term field studies of *C. w. obscurus* and *C. w. willardi*, histological examination of museum specimens, and literature records. Implications of harvest are considered and discussed in the context of life history and these reproductive data.

METHODS AND MATERIALS

I assessed reproductive condition of female *C. w. obscurus* during mark-recapture studies in the Animas Mountains, New Mexico (1990–1991 and 1993–1999), Peloncillo Mountains, Arizona and New Mexico (1995–1998), and Sierra San Luis, Sonora and Chihuahua, México (1998). Sampling spanned April through October but in most years was restricted to late summer and fall. I marked each individual using passive integrated transponders (Jemison et al., 1995) and recorded snout-vent length (SVL) and mass. Sex was determined by probing for hemipenes (Schaefer, 1934) and female reproductive status and number of embryos by palpation (Fitch, 1960). I used radio-telemetry to monitor nine pregnant females on a daily basis in the Animas (1994–1996) and Peloncillo mountains (1997). Transmitters (Holohil Inc., Carp, Ontario, Canada), weighing between 1.3 g and 2.8 g, and with average transmitting distance of 25–50 m, were surgically implanted in the body cavity (Reinert and Cundall, 1982). Isoflourane was used as an anesthetic. I also include similar observations of reproduction and mating in *C. w. willardi* provided by three independent research teams (see Acknowledgments) using similar methods at separate sites in the Huachuca Mountains, Cochise County, Arizona.

Additionally, 23 museum specimens were examined (Appendix A), including nine males (mean SVL = 460.2 ± 38.6 mm *SD*, range 406–544), nine females (mean SVL = 450.6 ± 27.1 mm *SD*, range 423–498), and a litter of five fully formed young taken from UAZ 27929. Specimens examined were collected in Arizona, New Mexico, Chihuahua, or Sonora between 1952–1994. The left testis, vas deferens, and part of the kidney were removed for examination. Tissues were embedded in paraffin and cut into sections at 5 µm. Slides were stained with Harris' hematoxylin followed by eosin counterstain. Testis slides were examined to determine the stage of the male cycle and *vas deferentia* were examined for sperm. Slides of kidney sexual segments were examined for secretory activity. *Vas deferentia* and kidneys were not available for examination from some road-killed males, thus six *vas deferentia* and seven kidneys were examined. Counts were made of enlarged follicles (> 8 mm length), oviductal eggs or embryos. Ovary slides were examined for the presence of secondary yolk deposition (*sensu* Aldridge, 1979a).

Statistics were calculated by hand or using BIOMstat vers. 3.30d (Applied Biostatistics, Inc., Port Jefferson, NY). For the purposes of this paper, "adult" refers to animals above minimum size for reproduction recorded herein. Symbolic codes for institutional collections follow Leviton et al. (1985).

RESULTS

Testicular histology was similar to that reported for two colubrid snakes, *Masticophis taeniatus* and *Pituophis catenifer* (Goldberg and Parker, 1975) and the viperid *Agkistrodon piscivorus* (Johnson et al., 1982). Only two stages were present in the male sample: recrudescence (renewal of spermatogenic cells characterized by

spermatogonial divisions with primary and secondary spermatocytes and occasional spermatids present) and spermiogenesis (metamorphosing and mature sperm present). Recrudescent males were identified from June (1) and August (1), while males undergoing spermiogenesis were found in June (1), July (3), September (1) and October (2). Sperm were present in all *vas deferentia* examined: June (2), July (2), August (1), and September (1). All kidney sexual segments were enlarged and contained secretory granules: June (2), July (2), August (1), September (1), and October (1). The shortest reproductively active male (spermiogenesis in progress) measured 406 mm SVL.

Events in the female reproductive cycle are summarized in Fig. 1. Histological examination of nine females revealed one undergoing yolk deposition (July), two with enlarged (> 8 mm diameter) follicles (August and September), three with developing young or embryos (April, June, and August), and three inactive (no yolk deposition; September and October). A total of 99 female *C. w. obscurus* were palpated in the field on 128 occasions (captures + recaptures). The shortest reproductively active female (histological material and all field studies) measured 402 mm SVL. Of 25 adult female *C. w. obscurus* examined May–July (the period when embryos can be detected by palpation but before parturition begins) 15 were pregnant. Using identical sampling period criteria, John Porter (*pers. comm.*) reported that eight of 20 adult female *C. w. willardi* examined between 1986 and 1996 were pregnant. Proportion of adult females pregnant (60% *C. w. obscurus*, 40% *C. w. willardi*, 51% pooled) versus not pregnant did not significantly differ from parity ($\chi^2 = 1.0$, $df = 1$, $P > 0.25$; $\chi^2 = 0.8$, $df = 1$, $P > 0.25$; $\chi^2 = 0.02$, $df = 1$, $P > 0.75$). Although I determined reproductive condition of individual adult females in consecutive years 12 times (Table 3), females were never found pregnant in consecutive

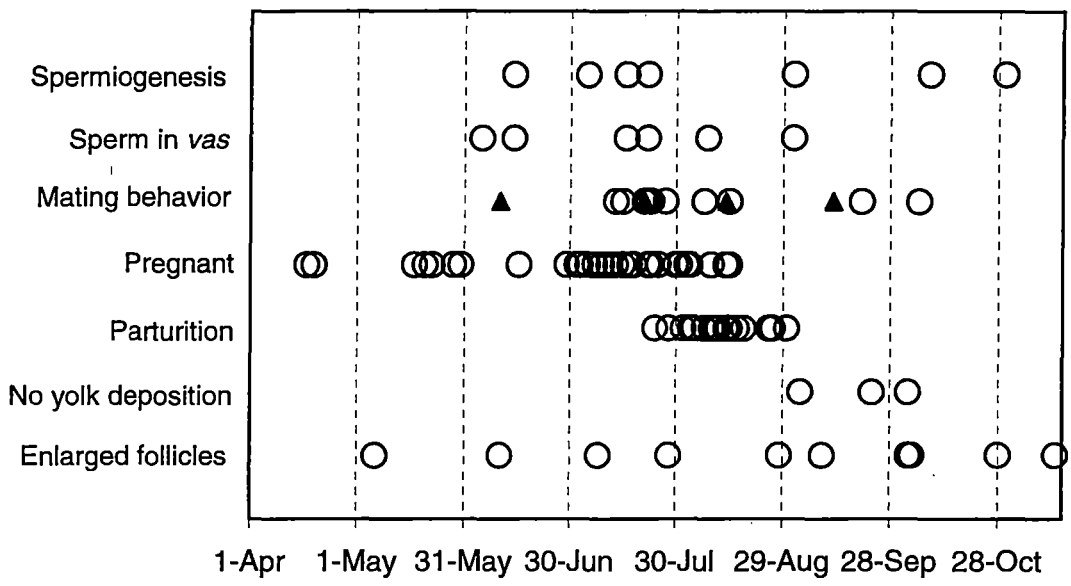

FIG. 1. Phenology of reproduction in wild *Crotalus willardi*. Records of "spermiogenesis", "sperm in *vas*", and "no yolk deposition" are based on histological observations alone. Records of "pregnant" and "enlarged follicles" are from histological and field observations. Records of "mating behavior" and "parturition" are from the literature and field observations. Under mating behavior open circles indicate copulation and closed triangles indicate courtship.

TABLE 3. Reproductive condition of individual adult female *Crotalus willardi* over an 11-year span. An N indicates years females were not pregnant, P indicates pregnancy, and blanks indicate reproductive condition was not determined that year. Identifiers beginning with CWW are *C. w. willardi* examined by John Porter in the Huachuca Mountains, Arizona and identifiers beginning with CWO are *C. w. obscurus* from the Animas Mountains, New Mexico.

Female	Year										
Identifier	86	87	88	89	90	91	92	93	94	95	96
CWW6	N	P		N	P		N				
CWW9	N	P		N	P						
CWW14		P		N			N				
CWW22							N	N	N	P	N
CWO70								N	P	N	P
CWO7E						P			P		P
CWO23										P	N

years. Several females were pregnant every other year, while other observations are suggestive of longer reproductive cycles. For example, one adult female was not pregnant for three consecutive years (Table 3).

Litter size (Appendix D) averaged 5.4 ± 1.6 *SD* (*N* = 25, range 2–9) across subspecies. Mean litter size of *Crotalus w. obscurus* was 5.5 ± 1.4 *SD* (*N* = 12, range 4–8), *C. w. silus* 4.0 ± 1.2 *SD* (*N* = 5, range 2–5), and *C. w. willardi* 6.3 ± 1.7 *SD* (*N* = 8, range 4–9). Low sample sizes and concomitant statistical power prohibits meaningful comparison of mean litter size among subspecies. Regression analysis revealed a significant positive relationship between ln(litter size) and ln(maternal SVL) among the 20 litters in Appendix D for which these data were available: (ln(litter size) = –14.64 + 2.66(ln(SVL)), $P < 0.01$, $r^2 = 0.36$). Back-transformed this regression equation describes the allometric relationship between the variables via a power function: litter size = $e^{-14.64}$ $SVL^{2.66}$. Neonatal morphometrics were not available for all litters reported in Appendix D. However, 42 neonates representing nine litters of *C. willardi* resulted in a mean SVL of 167.2 ± 7.4 mm *SD* (range 150–182) and 43 neonates representing nine litters gave a mean mass of 6.8 ± 1.2 g *SD* (range 5.0–9.0). Five litters of *C. w obscurus* averaged 164.3 ± 7.8 mm *SD* (*N* = 22, range 150–182) and 6.2 ± 0.9 g *SD* (*N* = 22, range 5.0–7.6), while three litters of *C. w. willardi* averaged 170.8 ± 5.7 mm *SD* (*N* = 15, range 160–180) and 7.2 ± 1.2 g *SD* (*N* = 16, range 5.4–9.0).

I never observed *C. w. obscurus* copulating in the field, but did find a male courting a female with eight enlarged follicles in the Peloncillo Mountains on 10 June. In the Huachuca Mountains my colleagues observed courtship in *C. w. willardi* on 21 July, 13 August, and 12 September and copulation on 13, 22 (*N* = 3), and 27 July, 7 and 14

August, 20 September, and 6 October. Prior to this report, the only published observation of *C. willardi* mating behavior in the wild was a pair of *C. w. silus* found copulating on 15 July in the Sierra de la Purica, Sonora, México (Armstrong and Murphy, 1979).

DISCUSSION

Although the sample of male *C. willardi* is too small to definitively describe the testicular cycle, the presence of males undergoing spermiogenesis from June–July and September–October suggests an aestival spermatogenesis in which spermiogenesis ends in September–October (*sensu* Saint Girons, 1982). This type of cycle is common in North American rattlesnakes and has been reported or inferred for *C. viridis* (Aldridge, 1979b), *C. tigris* (Goldberg, 1999a), *C. molossus* (Goldberg 1999b), *C. pricei* (Goldberg, 2000) and *Sistrurus catenatus edwardsii* (Goldberg and Holycross, 1999). The presence of mature sperm in the vas deferens of all snakes examined suggests males are capable of inseminating females throughout this period (June–September). All male *C. willardi* examined (June–October) had enlarged kidney sexual segments that contained secretory granules, a condition that typically coincides with breeding (Saint Girons, 1982). Because field observations and histological samples were strongly biased towards summer and fall, it is unclear if males are capable of inseminating females in the spring.

The presence of one female each in August and September with enlarged ovarian follicles (which would presumably ovulate the next spring) suggests northern *C. willardi* typically exhibit a biennial or longer reproductive cycle in which yolk deposition is completed in one or more reproductive seasons followed by a season in which ovulation, development of young and parturition occur. This view is corroborated by an absence of records for annual reproduction despite 12 opportunities for observation (Table 3.

Repeated observations of individual females over consecutive years (Table 3) demonstrate a capacity for biennial reproduction while other observations suggest more protracted reproductive cycles. For example, one adult female from the Huachuca Mountains was not pregnant for three consecutive years. Other field records are not inconsistent with a typically biennial or longer frequency of reproduction. A *C. w. obscurus* palpated 6 May 1999 (466 mm SVL, 90 g) did not contain yolked follicles but contained seven large follicles on 3 October 1999 (473 mm SVL, 147 g). Proportions of adult females pregnant vs. not pregnant did not significantly differ from parity, the expectation under a strictly biennial cycle. Approximately biennial or longer reproductive cycles are not uncommon in North American rattlesnakes as inferred from proportion of adult females pregnant in various studies (summarized in Seigel and Ford, 1987; Goldberg, 1999a, b). However, inferring frequency of reproduction from proportion of adult females breeding is controversial, as is the notion of rigidly biennial reproductive cycles (Blem, 1982; Seigel and Ford, 1987). Frequency of reproduction in nearctic vipers appears to be facultative and determined primarily by a combination of food availability, fat reserves, length of the active season, and population structure (Blem, 1982; Andren and Nilson, 1983; Seigel and Ford, 1987). Thus, there is considerable variation among and within species ranging from approximately annual to quadrennial reproductive cycles (Ernst, 1992). Likewise there is considerable variability within populations, such that in populations characterized by a generally biennial cycle some females reproduced in consecutive years (Diller and Wallace, 1984; Fitch and Pisani, 1993; Beaupre, 1995). Interestingly, two captive *C. w. willardi* females produced litters annually over four (litter size: 5, 6, 7, and 10) and three (litter size: 5, 5, and 1) consecutive years (McCrystal, H.

K., C. R. Schwalbe, and D. F. Retes. 1996. Unpubl. Rept. to Arizona Game and Fish Dept. Phoenix, Arizona). These observations demonstrate that *C. willardi* are physiologically capable of annual reproduction when freed from environmental constraints. However, these limited data suggest that such opportunities are probably rarely afforded under natural conditions for northern populations. Radio-tagged *C. willardi* substantially decrease both frequency and distance of surface movements during late October and early November (unpubl. data). Thus, to effect annual reproduction after parturition in August, wild females would have only ca. two to three months to complete yolk deposition before follicular development is arrested for the winter (as in *C. viridis*, Aldridge, 1979a). Histological observations of reproductively inactive (no yolk deposition) adult females in September (2) and October (1) suggest females may not even begin secondary yolk deposition until the summer following parturition. While it is possible that immediately postpartum females with substantial fat reserves and high autumnal foraging success might commence vitellogenesis prior to winter and complete it the following spring, it is unlikely that wild female *C. willardi* reproduce in consecutive years with any regularity. In light of these arguments the notion that *C. willardi* breeds annually (Bartlett and Tennant, 2000) requires qualification.

Young are born from late July throughout August, approximately one month (± 2 weeks) following the advent of summer rains and the appearance of juvenile *Sceloporus jarrovii* (Ballinger, 1973, 1979) and *S. virgatus* (pers. obs.). The diet of juvenile (< 350 mm SVL) *C. w. obscurus* consisted of 57.1% lizards (Holycross et al., *In press*) and 71% of these were identified as *Sceloporus* spp. Moreover, *Sceloporus* spp. were the only specifically identified prey found in juveniles under 278 mm SVL ($N = 10$). Centipedes

(*Scolopendra* spp.) constituted 33.3% of juvenile (< 350 mm SVL) diet (Holycross et al., *In press*) and may be more surface active during the monsoon period (ATH, pers. obs.). Parturition may be timed to coincide with this seasonal increase in food availability, a phenomenon observed in several colubrids (Godley, 1980; Seigel, 1984). In addition, increased rainfall, higher humidity, and altered diel thermal cycles associated with the summer rainy period may maximize survivorship of neonatal snakes that may be vulnerable to desiccation and rapid temperature changes.

Litter sizes described herein fall within the range of those previously recorded in the literature (2–9). Klauber (1972) gave litter sizes for *C. willardi willardi* (6 and 9) and *C. willardi silus* (2); neither localities nor female body sizes were provided. Lowe at al. (1986) stated that litter sizes are generally 4–6, range 2–9 for *C. willardi* in Arizona. Mean litter size reported here (5.4) is close to the modal litter size (7) reported for a large sample of snake species reviewed by Fitch (1970). Litter size increased allometrically with maternal SVL. Since abdominal volume increases as a cubic function of SVL, litter size is also expected to increase approximately cubically with maternal SVL (King, 2000). The observed regression coefficient ($b = 2.66$), or power coefficient in the back transformed equation, did not significantly differ from three (95% confidence interval = 0.9–4.4). Maternal SVL described 36% of the variation in litter size, which falls within the range of r^2 values (linear regression on untransformed litter size and length variables) compiled by Seigel and Ford (1987) for 13 populations representing eight species of Viperidae.

Observations of mating behavior in the wild are confined to June–October (Fig. 1). While the literature includes reports of captive *C. willardi* mating in April (Armstrong

and Murphy, 1979), June (Tryon, 1978), July (Martin, 1975a), August (Martin, 1976), September (Armstrong and Murphy, 1979), and even January (Armstrong and Murphy, 1979), these observations probably have little relevance to phenology under natural conditions. *Crotalus willardi obscurus* hibernate singly or in very small groups (ATH, pers. obs.; C. Painter, pers. comm.). If ovulation occurs prior to or upon egress, spring copulations may be unlikely since females are spatially dispersed and matings after ovulation are likely of no reproductive benefit (the ova may already be fertilized from female sperm stores). In addition females may not be receptive to spring courtship. Regardless, spring copulation by wild *C. willardi* remains undocumented.

Based on these data I postulate that parturition occurs late July through August and post-parturient females remain reproductively inactive prior to hibernation. The following year (or up to two years later) females begin secondary yolk deposition and mate in summer or fall. Ovulation and fertilization probably occur early the subsequent spring, followed by 4–5 months gestation. However, additional samples are necessary to determine the timing of ovulation.

Biennial or longer reproductive cycles and small litters (particularly for young females) suggest *C. willardi* has relatively low age-specific birth rates (m_x). Net reproductive rate (R_0; Gotelli, 1995) is a function of the product of m_x and age-specific survivorship (l_x) summed across ages (x). Simply defined R_0 is mean lifetime reproductive output of females. For iteroparous species that have relatively low realized natality and low juvenile survivorship, population persistence is largely dependent on adult survivorship, primarily adult females (high lifetime reproductive output). Thus, a conservative and prudent approach to management of sky island populations of *C.*

willardi requires continued protection from commercial harvest, as well as restriction of scientific harvest to males whenever possible.

Variation in the Diet of *Sistrurus catenatus* (Massasauga), with Emphasis on *S. c. edwardsii* (Desert Massasauga)

The Massasauga (*Sistrurus catenatus*), found from northern Chihuahua, México to southern Ontario, Canada, is threatened or endangered over much of its range and poses several interesting questions in phylogeography. Populations are highly fragmented (Gibbs et al., 1997) and there appear to be significant differences in natural history between Desert Massasauga (*S. c. edwardsii*) and other Massasauga populations. Although many aspects of the natural history of the Western Massasauga (*S. c. tergeminus*) and Eastern Massasauga (*S. c. catenatus*) have been researched (Wright 1941; Greene and Oliver, 1965; Keenlyne and Beer, 1973; Keenlyne, 1978; Reinert, 1981; Reinert and Kodrich, 1982; Seigel, 1986; Weatherhead and Prior, 1992; Johnson and Leopold, 1998; Seigel et al., 1998; Johnson, 2000), most aspects of the biology of *S. c. edwardsii* remain poorly studied (but see Hobert, 1997; Goldberg and Holycross, 1999). To conserve diversity within this group, a better understanding of population-specific variation in natural history is necessary (Greene, 1997).

Here I describe and compare diet of several populations of *S. c. edwardsii*, a form predominantly adapted to shortgrass prairies and desert grasslands, but occasionally found in dune formations and desert scrub (Degenhardt *et al.*, 1996). I also assess geographic variation in diet by comparing these results with quantitative studies of diet in populations of *S. c. catenatus* and *S. c. tergeminus*. Numerous anecdotal records (Appendix C) and four studies (Greene and Oliver, 1965; Keenlyne and Beer, 1973; Seigel, 1986; Hallock, 1991) provide an extensive dataset on diet of the two eastern subspecies. In contrast, there are very few published, explicit prey records for

S. c. edwardsii. Degenhardt et al. (1996) report a centipede (*Scolopendra* sp.) from the gut of a New Mexico specimen, Schwammer (1983) reports scavenging of a "grosse Maus" (big mouse) on a Colorado highway, and McKinney and Ballinger (1966) report *Uta* sp., unidentified lizard(s), and a centipede from four west Texas specimens. In addition, Hobert (1997) originally reported a portion of the data presented here (33 of 58 Colorado records) in an unpublished Master's thesis that was later summarized by Hammerson (1999).

MATERIALS AND METHODS

Prey were sampled from *S. c. edwardsii* during parallel autecological investigations in Arizona (1993–1997) and Colorado (1995–1998). The Arizona population inhabits tobosa (*Hilaria mutica*) grassland that blankets a volcanic cinder field in the San Bernardino and San Simon Valleys, Cochise County. *Sistrurus c. edwardsii* is found in 11 counties in southeastern Colorado (Hammerson, 1999) in shortgrass prairie dominated by blue gramma (*Bouteloua gracilis*) and buffalo grass (*Buchloe dactyloides*) growing on aridisols (Hobert, 1997). The majority (79%) of Colorado prey records were collected from a single population in Lincoln County; remaining records were from Cheyenne (7%), Crowley (4%), and Kiowa (10%) counties. I also collected samples from shortgrass prairie in the Rio Grande Valley, Valencia County, New Mexico (1997–1998). Although sampling efforts spanned April through October, in most years I emphasized late summer and fall sampling. I marked each live individual using passive integrated transponder tags (Jemison et al., 1995), recorded snout-vent length (SVL, mm) and mass (g), and determined sex by probing (Schaefer, 1934). I did not examine stomach contents of live snakes. Feces from live snakes (excreted voluntarily or palpated) and gut contents

of DOR animals were preserved in 70% ethanol or 10% formalin and subsequently identified. In addition, I include data gathered from Arizona, Colorado and New Mexico museum specimens (Appendix B) in the collections of the American Museum of Natural History (AMNH), Arizona State University (ASU), Museum of Southwestern Biology (MSB), Museum of Vertebrate Zoology (MVZ), University of Arizona (UAZ), University of Colorado Museum (UCM) and University of Northern Colorado Natural History Museum (UNC-MNH). Prey from specimens housed in captivity prior to preservation or which appeared to have been fed in captivity (allopatric or domestic prey) were omitted. All museum specimens from Arizona and Colorado that contained prey originated from the populations discussed above. Museum specimens from New Mexico that contained prey were from the Rio Grande River Valley (N = 8; Valencia, Bernalillo, Torrance, and Socorro Counties) or Mescalero Sands (N = 6; Chaves and Lea Counties).

Lizard remains were identified to genus, and to species when possible, from whole remains or by using a dichotomous key I constructed based on diagnostic scale characters for resident lizards. When possible, small mammals were identified to genus using characteristics (gross morphology, medulla configuration, and scale patterns) of dorsal guard hairs (Moore et al., 1974). Centipedes (*Scolopendra* spp.) were identified from exoskeletal remnants of chelicerae, leg or body segments. Snakes were identified from stomach remains and/or sections of skin found in the intestine. A single toad was identified to species.

For intra-subspecific statistical analyses I grouped prey into three classes: lizards, small mammals and centipedes. Two records, a snake and a toad, were excluded from analysis because they consisted of single observations. Rattlesnakes were divided into

two length classes, juveniles (< 300 mm SVL) and adults (≥ 300 mm SVL) based on approximate minimum size for sexual maturity (280 mm males, 329 mm SVL females; Goldberg and Holycross, 1999).

For inter-subspecific analyses I compared diet data from these populations of *S. c. edwardsii* with data for *S. c. tergeminus* from Missouri (Seigel, 1986) and Texas (Greene and Oliver, 1965; this study) and *S. c. catenatus* from Michigan (Hallock, 1991) and Wisconsin (Keenlyne and Beer, 1973). I omitted low frequency prey classes contributing zero values to cells and used an R x C test of independence to compare proportion of mammals vs. squamates. Aside from the following exceptions, all data in these samples were collected from single populations. The Colorado and New Mexico samples included a few samples from outside the focal population (see above). Hallock's (1991) sample was collected from museum specimens representing 17 counties in Michigan. Greene and Oliver's (1965) study included 21 prey identified from specimens collected in Parker and Tarrant counties and two additional records. I augmented Greene and Oliver's (1965) Texas sample of *S. c. tergeminus* by examining 161 specimens collected in the intervening years and deposited in the University of Texas at Arlington (UTA) collection (Appendix B). These specimens yielded 88 additional prey records. Most prey were from Parker and Tarrant counties ($N = 78$) with remaining prey records from Clay ($N = 1$), Haskell ($N = 1$), Hood ($N = 3$), Johnson ($N = 2$), Stonewall ($N = 1$), Throckmorton ($N = 1$), and Wilbarger ($N = 1$) counties.

Statistics were computed using BIOMstat version 3.3 (Rohlf and Slice, 1999) or a pocket calculator. Means are reported ± 1 *SE*. Nominal significance level was set at $\alpha = 0.05$ and Dunn-Šidák adjusted (Sokal and Rohlf, 1995) for multiple tests of the same data

set using the same test statistic (Cabin and Mitchell, 2000). Specifically, for four R x C tests of independence using all prey records $\alpha' = 0.013$. Parametric statistics were only applied to normally distributed data. Institutional abbreviations follow recommendations of Leviton *et al.* (1985).

RESULTS

In Arizona and New Mexico I recorded 155 field encounters (captures + recaptures + DOR: 132 Arizona, 23 New Mexico) with 146 individual *S. c. edwardsii* (124 Arizona, 22 New Mexico). Seventy-one (63 Arizona, 8 New Mexico) of these encounters yielded 84 identifiable prey (75 Arizona, 9 New Mexico). In Colorado, 32 of 80 snakes encountered DOR (1995–1998) contained remains of 33 identifiable prey, and feces collected from 21 live specimens (1995–1996) yielded 23 prey. Twenty-three (36%) of 64 museum specimens examined (exclusive of those from collected in this study) yielded 25 identifiable prey (9 Arizona, 2 Colorado, 14 New Mexico).

From the three populations of *S. c. edwardsii*, I identified remains of 97 lizards (58.8%), 51 mammals (30.9%), 15 centipedes (9.1%), one toad (0.6%, *Spea bombifrons*, Colorado) and one snake (0.6%, *Tantilla nigriceps*, central New Mexico). Identifications of lizards and mammals are provided in Table 4. All 15 centipedes were identified as *Scolopendra* spp., only four of which were found in a sample containing other prey remains. Judging by orientation in the stomach, six of eight centipedes were swallowed headfirst, whereas direction of ingestion could not be determined for two. All 18 lizards found in stomachs were swallowed headfirst. I detected remains of two prey species in 16 snakes: lizard + mammal ($N = 7$), lizard + lizard ($N = 4$), centipede + mammal ($N = 3$), centipede + lizard ($N = 1$) and mammal + mammal ($N = 1$). One Arizona snake

(ASU30149) contained three recently ingested lizards in its stomach (*Holbrookia maculata*, *Sceloporus undulatus* and *Cnemidophorus uniparens*). Because I used prey remains from feces and the intestine (in addition to stomach contents) it is possible that snakes with multiple prey of the same prey type were overlooked. Of the 165 *S. c. edwardsii* prey identified, 60 were identified from remains in the stomach and 105 from the colon or feces.

Predator SVL significantly differed among prey categories (Kruskal-Wallis test, $H_{(2)} = 7.90$, $P = 0.01$; Fig. 2A). Snakes that ate mammals were longest (mean SVL = 362 ± 7 mm, $N = 51$), followed in turn by those that fed on centipedes (349 ± 12 mm, $N = 15$) and lizards (329 ± 8 mm, N = 95). Snakes that fed on *Cnemidophorus* spp. ($N = 34$), *Sceloporus* spp. ($N = 33$) and *Holbrookia maculata* ($N = 19$) did not significantly differ in SVL (Kruskal-Wallis test, $H_{(2)} = 2.13$, $P = 0.34$). Juveniles and adults contained similar proportions of centipedes, but juveniles contained significantly more lizards and fewer mammals ($G_{\text{Williams}} = 10.8$, $df = 2$, $P < 0.01$; Table 5). Diet was independent of sex ($G_{\text{Williams}} = 0.7$, $df = 2$, $P = 0.70$; Table 5) and source (stomach vs. colon/feces) of sample ($G_{\text{Williams}} = 5.8$, $df = 2$, $P = 0.06$; Table 5). Centipedes and mammals appear to be taken more frequently later in the foraging season (Fig. 3). Proportion of prey classes consumed by *S. c. edwardsii* differed among populations ($G_{\text{Williams}} = 14.0$, $df = 4$, $P = 0.007$; Table 5). However, all pair-wise comparisons (AZ-CO, AZ-NM, CO-NM) between populations comprised non-significant subsets ($G = 1.7$, 10.3, 11.7 respectively) of this analysis. The small New Mexico sample contained proportionately more centipedes and fewer mammals than the other two samples (Table 5).

From the Texas *S. c. tergeminus* I examined, I identified remains of 70 mammals (79.5%), 10 lizards (11.4%), six snakes (6.8%) and two birds (2.3%). Mammals consisted of 39 soricids (four identified as *Cryptotis parva*), 13 cricetids, two heteromyids (*Perognathus* spp.), one geomyid, and 15 unidentified further. Lizards consisted of five *Cnemidophorus* spp. and five unidentified skinks. One snake was identified as *Tropidoclonion lineatum*. I detected remains of two prey species in five snakes: mammal + mammal ($N = 2$), snake + mammal ($N = 2$), and lizard + mammal ($N = 1$). Orientation of prey remains in the stomach suggested that 18 mammals, three *Cnemidophorus* spp., three skinks (identified from tails in the stomach), two snakes, and one bird were consumed headfirst whereas one mammal was consumed rump first. Proportion of prey classes for this population as well as other previously published diet studies of the two eastern subspecies are provided in Table 6.

In comparisons among subspecies I found that proportion of mammals vs. squamates was dependent on source population ($G_{\text{Williams}} = 120.8$, $df = 6$, $P \approx 0$). The Michigan, Missouri and Wisconsin samples comprised a non-significant subset of this analysis ($G = 4.3$), as did the Arizona, Colorado, and New Mexico samples ($G = 3.3$). Interestingly, the Michigan, Missouri and Texas populations also comprised a non-significant subset ($G = 1.9$). Snake SVL did not significantly differ between *S. c. edwardsii* (pooled) and *S. c. tergeminus* (Texas specimens examined in this study) that ate squamates (ANOVA, $F_{(1,110)} = 0.35$, $P = 0.56$) or mammals (ANOVA, $F_{(1,119)} = 0.89$, $P = 0.35$). Nevertheless, proportion of squamates vs. mammals consumed by 300–400 mm SVL *S. c. edwardsii* and 300–400 mm SVL *S. c. tergeminus* from Texas was dependent on subspecies ($G_{\text{Williams}} = 31.9$, df = 1, $P < 0.01$; Fig. 2).

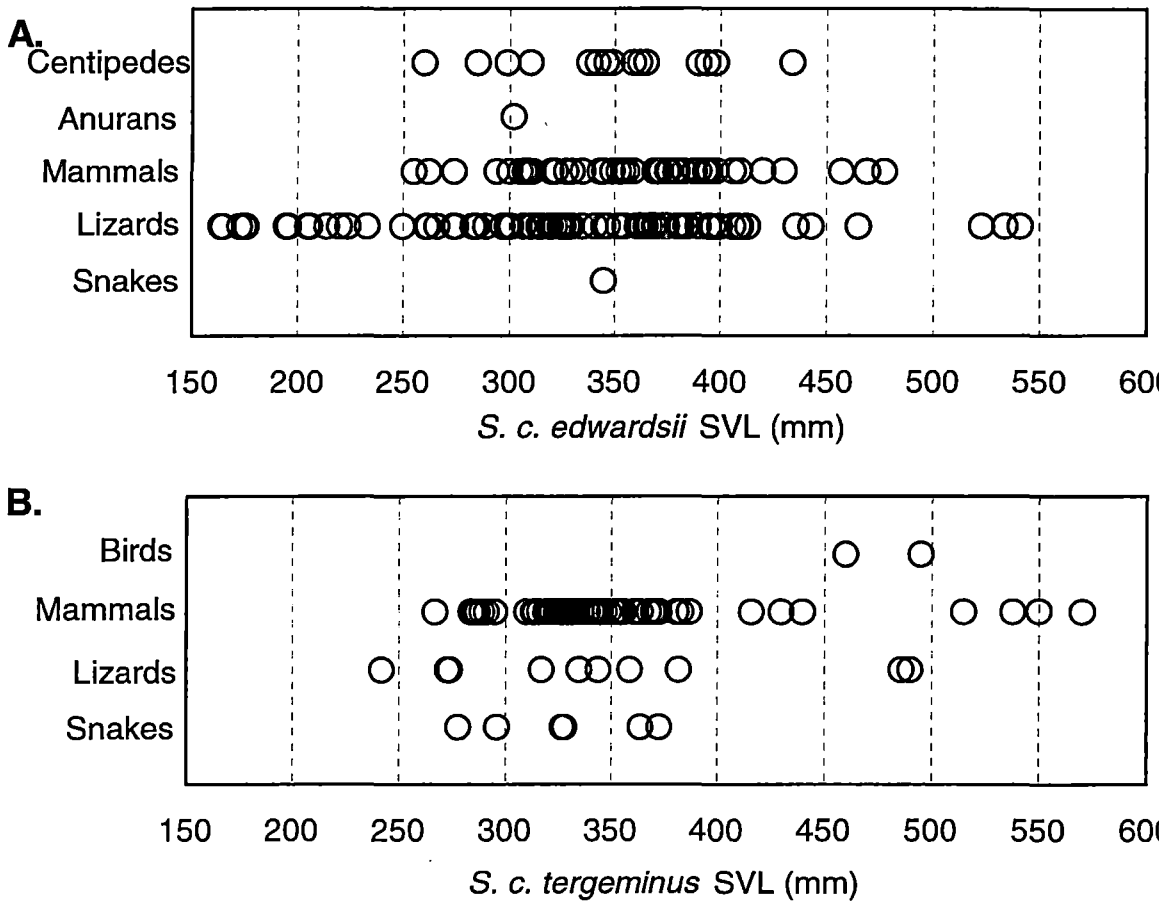

FIG. 2.Association between prey class and predator snout-vent length for A.) *S. c. edwardsii* from Arizona, New Mexico, and Colorado (N = 163), and B.) *S. c. tergeminus* from Texas (N = 88).

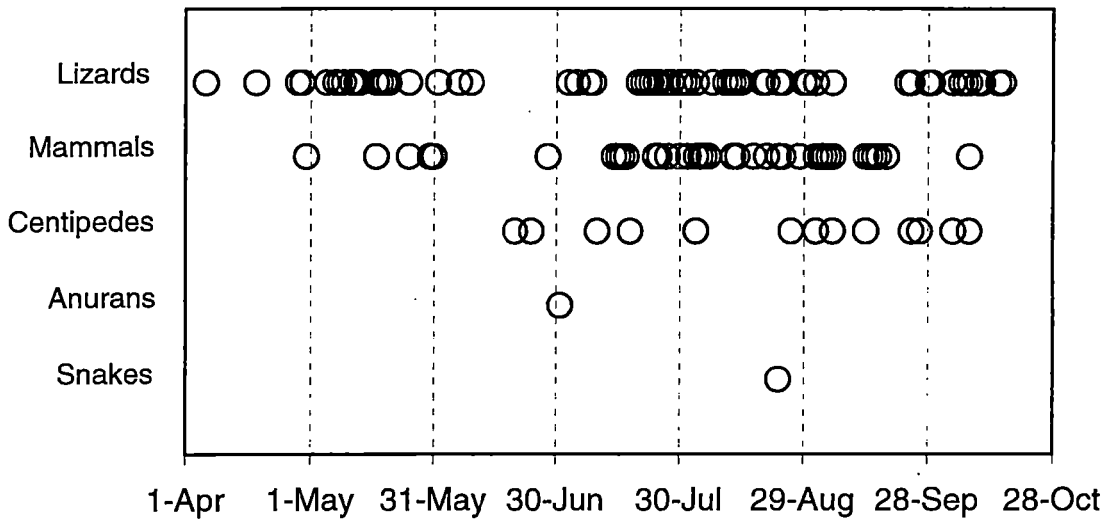

FIG. 3. Seasonal distribution of prey consumed by *S. c. edwardsii* (N = 165).

TABLE 4. Prey consumed by *Sistrurus catenatus edwardsii* in this study.

Prey taxon	*f*	(%)
ARTHROPODA	**15**	**(9.1)**
Scolopendra spp.	15	(9.1)
Anura	**1**	**(0.6)**
Spea bombifrons	1	(0.6)
MAMMALIA	**51**	**(30.9)**
Baiomys taylori	1	(0.6)
Notiosorex crawfordi	3	(1.8)
Onychomys leucogaster	1	(0.6)
Perognathus spp.	1	(0.6)
Perognathus flavescens	8	(4.9)
Peromyscus spp.	1	(0.6)
Reithrodontomys megalotis	8	(4.9)
Unidentified mammal	28	(17.0)

continued

TABLE 4, continued

Prey taxon	*f*	(%)
SQUAMATA	**98**	**(59.4)**
Tantilla nigriceps	1	(0.6)
Cnemidophorus spp.	6	(3.6)
Cnemidophorus sexlineatus	3	(1.8)
Cnemidophorus uniparens	25	(15.2)
Holbrookia maculata	20	(12.1)
Eumeces obsoletus	2	(1.2)
Sceloporus spp.	4	(2.4)
Sceloporus undulatus	29	(17.6)
Urosaurus ornatus	1	(0.6)
Uta stansburiana	2	(1.2)
Unidentified lizard	5	(3.0)
Total	**165**	**(100)**

TABLE 5. Relative occurrence of *Sistrurus catenatus edwardsii* prey class by state, sex, age class and sample type (N = 163 for each comparison). Sample size for age class is lower (N = 161) because two specimens were not measured. One anuran and one snake are omitted (see text).

	N	Centipedes	Lizards	Mammals
		f (%)	f (%)	f (%)
AZ	84	5 (5.6)	54 (64.3)	25 (29.8)
CO	57	3 (5.3)	31 (54.4)	23 (40.4)
NM	22	7 (31.8)	12 (54.6)	3 (13.6)
Males	95	8 (8.4)	53 (55.8)	34 (35.8)
Females	68	7 (10.3)	42 (61.8)	19 (27.9)
Juveniles	36	3 (8.3)	29 (80.6)	4 (11.1)
Adults	125	12 (9.6)	66 (52.8)	47 (37.6)
Colon	58	8 (13.8)	38 (65.5)	12 (20.7)
Stomach	105	7 (6.7)	59 (56.2)	39 (37.1)

TABLE 6. Relative occurrence of *Sistrurus catenatus* prey class by population. Arizona (AZ), Colorado (CO), and New Mexico (NM) populations are *S. c. edwardsii*, Texas (TX) and Missouri (MO) populations are *S. c. tergeminus* and Michigan (MI) and Wisconsin (WI) populations are *S. c. catenatus*.

		Mammals		Lizards		Snakes		Centipedes		Other		Source
	N	*f*	(%)	*f*	(%)	*f*	(%)	*f*	(%)	*f*	(%)	
AZ	84	25	(29.8)	54	(64.3)	0		5	(6.0)	0		A
CO	57	23	(39.7)	31	(53.5)	0		3	(5.2)	1	(1.7)	A
NM	22	3	(13.0)	12	(52.2)	1	(4.4)	7	(30.4)	0		A
MO	20	16	(80.0)	0		4	(20.0)	0		0		B
TX	111	81	(73.0)	15	(13.5)	11	(9.9)	0		4	(3.6)	C (*N* = 23); A (*N* = 88)
MI	43	33	(76.4)	0		6	(14.0)	0		4	(9.3)	D
WI	96	85	(93.4)	0		5	(5.5)	0		1	(1.1)	E

A. This study.

B. Seigel, 1986

C. Green and Oliver, 1965

D. Hallock

E. Keenlyne and Beer, 1973

DISCUSSION

Based on a few records from *S. c. catenatus* (Appendix C), several early works erroneously suggest that frogs are prominent in the diet of *S. catenatus* (Ditmars, 1912; Evermann and Clark, 1914; Pope, 1926; Ruthven *et al.*, 1928), an assertion later reiterated by Wright and Wright (1957) and Klauber (1972). Subsequent quantitative studies (see Table 6) suggest that although anurans are preyed upon, they are not consumed in abundance by any subspecies of *S. catenatus*.

These early reports on eastern subspecies may have led Fowlie (1965) to state that *S. c. edwardsii* feed primarily on amphibians. Though Fowlie based his conclusion on "examination of stomach contents", he neither cited a source nor referenced specimens. Despite the paucity of published prey records for *S. c. edwardsii*, several authors (Lowe et al., 1986; Greene, 1997) correctly postulate a diet consisting primarily of mice and lizards but do not provide data. Several regional field guides (Stebbins, 1985; Bartlett and Tennant, 2000) list mice and lizards as food of *S. c. edwardsii*, but still include frogs, and fail to mention centipedes, as important components of diet. These data demonstrate lizards are the modal prey of *S. c. edwardsii*, though small mammals and centipedes also comprise significant proportions of the diet.

Although *S. catenatus* appears to consume anurans infrequently, Werler and Dixon (2000) suggest toads may "constitute an important part of the Massasauga's diet when they are available" based on *S. catenatus'* large adrenal glands (Smith and White, 1955). In other ophidians, enlarged adrenals apparently relate to a diet of toads (Smith and White, 1955), many of which have toxic skin-gland secretions containing epinephrine and digitaloid compounds. Interestingly, this speculation is based on

examination of the adrenal glands of *S. c. catenatus* (Smith and White, 1955) for which there are records of frog (but not toad) consumption (Appendix C). Certainly both bufonid and pelobatid toads are a syntopic and seasonally abundant potential food source throughout the range of *S. c. edwardsii*. However, the majority of specimens were collected during rainy seasons when toads were surface-active and abundant, yet I documented only a single toad among the 60 prey identified from stomach contents.

Lizards comprise a surprisingly large proportion of the adult diet of *S. c. edwardsii* relative to the diet of conspecifics and to the prevalence of mammals in the diet of many northern pit vipers (Mushinsky, 1987; Ernst, 1992). Ontogenetic shifts in diet account for prevalence of lizards in the diet of some rattlesnakes (*e.g.*, Mackessy, 1988; Holycross *et al.*, *in press*), but only partially explain their prevalence in the diet of *S. c. edwardsii*. Clearly, *S. c. edwardsii* < 250 mm SVL feed exclusively on lizards, probably because these snakes are physically incapable of ingesting even small rodents. However, squamates (lizards) are consumed 1.5 times more often than mammals even after these gape-limited predators exceed 300 mm SVL and begin to consume a variety of small mammals (Fig. 2A).

The high number of solitary centipede records suggests directed foraging on live centipedes rather than secondary ingestion or scavenging. Large centipedes are not uncommon in the diets of *Sistrurus miliarus* (Hamilton and Pollack, 1955), *Crotalus enyo*, (Taylor, 2001), *Crotalus willardi obscurus* (Holycross *et al.*, *in press*), and *Crotalus lepidus klauberi* (ATH, unpubl. data). Although foraging behaviors associated with mammalian prey have been studied extensively, very little is known of how rattlesnakes forage on centipedes. Nevertheless, it seems likely that centipede-eaters have

evolved specific adaptations for foraging on this fractious and venomous prey. For example, Rubio (1998) wondered if centipede-eaters are resistant to centipede venom. Several observations suggest centipede-specific prey-handling behaviors in *C. lepidus*, *C. willardi*, and *S. c. edwardsii* (Rubio, 1998; H. McCrystal, *pers. comm.*; D. Sias, *pers. comm.*.). Regardless of how centipedes are envenomated and handled, these few observations hint that *S. c. edwardsii* usually ingests them headfirst.

Diet of the three *S. c. edwardsii* populations is essentially homogeneous in both intra- and inter-subspecific comparisons. Not only do *S. c. edwardsii* populations consume similar proportions of broadly defined taxa, but they also consume many of the same prey genera and species (*e.g.*, *H. maculata*, *S. undulatus*, *Cnemidophorus* spp.). Likewise, Wisconsin and Michigan *S. c. catenatus* and Missouri *S. c. tergeminus* populations all consume similar proportions of mammals vs. squamates and consume similar prey genera. Thus, similarities within and differences among the diet of these eastern and western groups are not limited to proportion of mammals vs. squamates consumed but extend to the taxa of mammals and squamates consumed. Populations in Arizona, Colorado and New Mexico appear to rely primarily on harvest mice (*Reithrodontomys* spp.) and pocket mice (*Perognathus* spp.) while Michigan, Wisconsin and Missouri populations rely chiefly on voles (*Microtus* spp.) and shrews (*Blarina* spp. and *Sorex* spp.) and occasionally jumping mice (*Zapus* spp.). Correspondingly, Arizona, Colorado and New Mexico (*S. c. edwardsii*) populations rely heavily on lizards and rarely eat snakes, whereas snakes are the only squamates documented in the diet of Michigan, Wisconsin, and Missouri populations and were usually consumed by juveniles (Keenlyne and Beer, 1973; Seigel, 1986; Hallock, 1991). The Texas population of *S. c.*

tergeminus did not significantly differ from the Michigan and Missouri populations in proportion of mammals vs. squamates consumed, and likewise consumed a high proportion of shrews. Infrequent consumption of ranid frogs and the absence of centipedes also suggest primary dietary affiliations with eastern populations. The Texas sample of squamates consisted of similar proportions of snakes and lizards (Table 6). Hence, the Texas population appears intermediate between the divergent diets of eastern and western groups, but appears to have more commonalities with eastern diets.

Differences in diet among populations of *S. c. edwardsii*, *S. c. tergeminus* and *S. c. catenatus* parallel other ecological and behavioral differences. *Sistrurus c. edwardsii* inhabit dry grasslands and dune formations (Stebbins, 1985; Degenhardt *et al.*, 1996; Hammerson, 1999), whereas *S. c. tergeminus* and *S. c. catenatus* typically inhabit wet grasslands, marshes, swamps, and small meadows in woodlands (Seigel, 1986; Reinert and Kodrich, 1982; Weatherhead and Prior, 1992). Prey of eastern subspecies are generally associated with mesic habitats (Appendix C) whereas *S. c. edwardsii* prey are associated with xeric grassland communities (Table 4). *Sistrurus c. tergeminus* in Parker and Tarrant Counties, Texas occupy intermediate habitat described as "gently rolling, tall grass prairie, interrupted by an occasional shallow creek or rocky hillside" (Greene and Oliver, 1965).

Clearly, geographic variation in prey availability and clinal variation in size (with eastern forms larger than western forms) account, in part, for geographic variation in diet. Lizards and large centipedes are not abundant in the habitat of some eastern populations, and larger size allows eastern subspecies to consume a wider size-range of small mammals. However, *S. c. edwardsii* and *S. c. tergeminus* that ate the same prey class

(mammals or squamates) did not significantly differ in SVL. In this comparison, size alone does not account for the significant disparity in proportion of prey classes consumed. The Texas sample of *S. c. tergeminus* > 300 mm SVL consumed mammals 5.5 times more often than squamates (Fig. 2B), despite the presence of a fairly abundant lizard fauna. Whereas *S. c. edwardsii* > 300 mm SVL consumed squamates 1.5 times more often than mammals (Fig. 2A), despite a diverse and abundant rodent fauna at all three field sites. Thus, differences in diet may also reflect foraging adaptations to different prey communities rather than opportunism alone, as suggested by studies of the stimulus-control of foraging behaviors. For example, Schuett *et al.* (1984) reported that captive juvenile *S. c. catenatus* caudal lure for frogs and Reiserer (*In press*) reports that captive juvenile *S. c. tergeminus* from Kansas caudal lure for frogs but not lizards. Conversely, Reiserer (*In press*) reports that juvenile *S. c. edwardsii* from Arizona caudal lure for lizards but not frogs. Reiserer's experiments suggest the primary stimulus-control is prey movement patterns. Analysis of venom proteins from Arizona, Colorado, Kansas, Missouri, New Mexico and Wisconsin populations group *S. c. edwardsii* separate from conspecific subspecies (Milne and Mackessy, *in prep.*). Venom from *S. c. edwardsii* is also consistently more toxic (to inbred lab mice, and presumably to lizards) than venom from the two eastern subspecies (S. Mackessy, *pers. comm.*). The evolution of a switch in stimulus-control of a foraging behavior and differences in venom profiles among subspecies suggest adaptation to different prey communities. Other aspects of foraging ecology may have undergone similar adaptive radiation. Rattlesnakes rely heavily on chemical cues when selecting ambush sites. *Sistrurus miliarius barbouri* prey primarily on frogs and select foraging sites based on the presence of frog odors (Roth et al., 1999),

whereas *Crotalus viridis viridis* select sites based on the presence of rodent olfactory cues (Duvall et al., 1990a, 1990b). I imagine that experiments examining foraging site selection by *S. catenatus* using different prey odors would yield patterns (among subspecies) similar to Reiserer's experiments on stimulus-control for caudal luring.

Though the precise pattern and tempo of these evolutionary changes is unclear, paleoecological evidence considered in light of extant patterns in natural history offers intriguing insights. Fossil remains from the middle and late Pliocene (Kansas, Nebraska and Texas) and the middle Pleistocene (Kansas and Nebraska) suggest *S. catenatus* was present over much of its current range in the Great Plains both prior to and during Pleistocene glacial oscillations (Holman, 2000). Schmidt (1938) argued that most of the range of *S. c. catenatus* is the product of an eastward expansion across the "prairie peninsula" of the Great Lakes region following the retreat of the Wisconsinan Laurentide Ice Sheet. However, a fossil *S. catenatus* from eastern West Virginia (Holman, 2000) precedes the Laurentide Ice Sheet by ca. 300,000 years or more, and raises the possibility that during the Wisconsinan an isolated group of *S. catenatus* persisted southeast of the ice sheet. Thus a deeper divergence may separate western forms from their eastern relatives and occupation of the "prairie peninsula" may not have originated from the west alone. Regardless of intraspecific phylogeny, *S. c. catenatus* and *S. c. tergeminus* appear to have retained adaptations to pluvial communities, whereas populations of *S. c. edwardsii* appear to have derived adaptations to xerification of western habitats and prey communities during the Holocene. Pluvial communities in the southwest probably approximated modern conditions for *S. c. tergeminus* and *S. c. catenatus* in that the region was wetter and cooler. Indeed, the most southwestern populations of *S. c.*

edwardsii persist in desert grasslands devoid of microtine rodents, yet as recently as ca. 4,000–9,000 years ago this area supported mesic grasslands and lakeshores that teemed with microtines (Van Devender and Worthington, 1977; Van Devender, 1995). If populations of *S. c. edwardsii* were isolated from one another early in this history they may have convergently derived adaptations to xerification and new prey communities (*e.g.*, stimulus-control of feeding responses, venom characteristics, habitat use, body size and its correlates).

Until a better understanding of this group's phylogeography is achieved, prudent conservation practices will recognize the uniqueness of *S. c. edwardsii* and afford it separate status in risk assessment and management. These populations currently depend on open grassland habitats that support a fauna of grassland lizards, large centipedes, and small mammals. Management practices that negatively affect these communities and/or prey species will undoubtedly negatively affect *S. c. edwardsii*. Desertification (anthropogenic by definition) and the spread of agriculture have already led to the extirpation of populations in Arizona (Lowe et al., 1986).

Foraging Ecology of a Threatened Rattlesnake, *Crotalus willardi obscurus.*

Predator-prey interactions are widely recognized as primary influences in snake evolution (Greene, 1983; Mushinsky, 1987). Selective pressures as they relate to diet affect morphology (Arnold, 1993, Forsman and Shine, 1997), physiology (Secor and Diamond, 1998), community structure (Reynolds and Scott, 1982; Vitt, 1983; Rodríguez-Robles and Greene, 1996), movement/activity patterns (Duvall et al., 1990b; Secor, 1995; Madsen and Shine, 1996) and habitat use (Reinert et al., 1984; Chandler and Tolson, 1990). Ontogenetic, sexual, or geographic differences in diet may reflect associated variation in other aspects of autecology and/or phylogeographic history. Thus, dietary assays can provide valuable insights into the evolution of natural and life history characters (Shine, 1996; Rodríguez-Robles et al., 1999); insights that may have significant implications for conservation of imperiled populations (e.g., Downes and Shine, 1998; Shine et al., 1998). Unfortunately, data are often lacking or difficult to obtain for endangered species, particularly those that are secretive.

Here I describe and evaluate the foraging ecology of the federally threatened (U.S. Fish and Wildlife Service, 1978) New Mexico Ridgenose Rattlesnake, *Crotalus willardi obscurus*, and discuss autecological insights as well as implications for conservation. *Crotalus willardi* is presumed to be "predominantly adapted to montane pine-oak woodlands" of the Sierra Madre Occidental (McCranie and Wilson, 1987), though its niche in this community is poorly understood. *Crotalus w. obscurus* is restricted to three isolates inhabiting the Animas Mountains (New Mexico, USA), Peloncillo Mountains (Arizona and New Mexico, USA) and Sierra San Luis (Sonora and Chihuahua, México). Prior to this study, a single natural prey record existed for this

subspecies (Marshall, 1957), although some of the data presented herein were summarized in Degenhardt et al. (1996).

METHODS AND MATERIALS

Crotalus w. obscurus was sampled throughout its range during an investigation of its autecology and conservation biology. Mark-recapture sampling took place in the Animas Mountains (1990–1991 and 1993–1999), Peloncillo Mountains (1995–1998) and Sierra San Luis (1988–1989 and 1998). Observations of foraging behavior were effected through unobtrusive daily monitoring of radio-tagged snakes in the Animas Mountains (1994–1996 and 1999) and Peloncillo Mountains (1997). Although sampling efforts spanned April through October, in most years they were restricted to late summer and fall (July–August). I marked each individual using passive integrated transponders (Jemison et al., 1995), recorded snout-vent length (mm) and mass (g) and determined sex by probing (Schaefer, 1934). I attempted to collect fecal samples from all snakes (regardless of whether remains were detected in the colon) by gently massaging the distal abdomen in the direction of the vent. Fecal material was preserved in 70% ethanol or 10% formalin for subsequent identification. Presence or absence of prey in the stomach was determined by palpation. Rarely, stomach contents were gently massaged to the mouth of the snake for identification and returned to the stomach by the same method. I did not attempt to identify stomach contents if injury to the snake seemed possible based on predator/prey size ratio or presumed type of prey (e.g. *Sceloporus* spp. which are ingested head-first due to the orientation of their spiny scales). Lizard remains were identified to genus and when possible species, using a dichotomous key based on diagnostic scale characters for resident lizards. Small mammals were identified to genus using characteristics (gross

morphology, medulla configuration and scale patterns) of dorsal guard hairs (Moore et al., 1974). Centipedes (*Scolopendra* spp.) were identified from exoskeletal remnants of chelicerae, leg, or body segments. Birds were identified from diagnostic primary flight feathers and tarsi. Also included in these analyses is a specimen (UAZ 27943) collected in the Sierra San Luis on 3 September 1952 (Marshall, 1957).

A survey of the small mammal community at the Animas Mountain study site (ca. 2100–2600 m elevation) was conducted on 16 nights in July and August 1996. Sherman live-traps baited with oatmeal/birdseed mixture were concurrently set in lines of 25 traps each in four habitats: pine-oak woodland (*Pinus engelmanni*, *Pinus strobiformis*, *Pseudotsuga menziesii* and *Quercus gambelli*), encinal (*sensu* Lowe, 1967:52–53; *Quercus arizonica*, *Quercus emoryi*, *Quercus hypoleucoides*, *Juniperus deppeana* and *Pinus cembroides*), talus slope (*Quercus rugosa* and *Robinia neomexicana* interspersed) and grass-dominated plateau (*Eragrostis intermedia*, *Bouteloua gracilis*, *Muhlenbergia trifida*). Total trapping effort equaled 1,600 trap-nights.

For statistical analyses, I grouped prey into four classes: small mammals, lizards, centipedes and birds. I divided snake foraging season into two periods, dry season (18 April–15 July) and wet season (16 July–24 October). Snakes < 350 are referred to as juveniles while animals ≥ 350 mm SVL are referred to as adults. Holycross and Goldberg (2001) report that the shortest reproductive male examined measured 406 mm SVL, the shortest pregnant female measured 402 mm SVL and several females < 425 mm SVL were pregnant. Pregnant females in these populations are in the second year of their first reproductive cycle (Holycross and Goldberg, 2001). Given this evidence and the relatively small sample of histological examinations (Holycross and Goldberg, 2001), I

believe it reasonable to assume that wild animals reach reproductive maturity between 350–400 mm SVL.

Statistics were computed using BIOMstat version 3.3 (Rohlf and Slice, 1999). Significance level for individual tests was Bonferroni adjusted where appropriate. Institutional abbreviations are as listed in Leviton et al. (1985).

RESULTS

Radio-tagged snakes were observed on 1,951 occasions during the course of the study. I recorded 317 encounters (captures + recaptures: 230 Animas, 69 San Luis, 18 Peloncillo) with 246 individual *C. w. obscurus* (160 Animas, 69 San Luis, 17 Peloncillo). Eighty-nine (28.1%) encounters yielded 95 identifiable prey items, 88 identified from feces and seven from stomach contents. Five fecal samples contained remains of two prey species and one individual contained prey in its stomach and feces. Three individuals contained identifiable prey on two separate encounters. Presence/absence of prey was independent of sex ($G = 1.21$, $df = 1$, $P = 0.271$) but dependent on length class ($G = 5.04$, $df = 1$, $P = 0.025$), with juveniles containing prey more frequently (34.5%) than adults (24.4%).

Small mammals (38.9%) and lizards (40.0%) comprised the majority of prey consumed (Table 7). *Peromyscus* spp. constituted 64.9% of rodents consumed and *Sceloporus* spp. accounted for 68.4% of lizards. I specifically identified four of the

TABLE 7. Prey consumed by *Crotalus willardi obscurus*. Numbers in parentheses indicate percent of total prey by major taxonomic grouping (Arthropoda, Aves, Mammalia, Squamata).

Prey taxon	Frequency	Percent total number prey	Source
ARTHROPODA		(15.8)	
Scolopendra spp.	15	15.8	This study
AVES		(5.3)	
Myadestes townsendi	1	1.0	This study (MSB 21341)
Wilsonia pusilla	1	1.0	Marshall, 1957
Unidentified bird	3	3.2	This study
MAMMALIA		(38.9)	
Sorex spp.	2	2.1	This study
Perognathus spp.	1	1.0	This study
Peromyscus spp.	24	25.3	This study
Reithrodontomys spp.	1	1.0	This study
Unidentified mammal	9	9.5	This study
SQUAMATA		(40.0)	
Sceloporus spp.	26	27.4	This study
Cnemidophorus spp.	8	8.4	This study
Unidentified lizard	4	4.2	This study
Total	95	100.0	

Peromyscus spp. as *P. boylii* and six of the *Sceloporus* spp. as *S. jarrovii* (Table 7). Seven of the *Cnemidophorus* spp. were obtained from Sierra San Luis specimens and one from a Peloncillo specimen. All 15 centipedes were identified as *Scolopendra* spp., only one of which was found in a sample containing other prey (lizard) remains.

Predator SVL significantly differed among prey classes (Kruskal-Wallis test, $H_{(3)} = 31.8$, $P < 0.001$; Fig. 4). Snakes that ate birds were longest (mean SVL = 450 ± 52.2 mm, $N = 5$), followed by those that ate small mammals (mean SVL = 422 ± 64.2 mm, $N = 37$), lizards (mean SVL = 343 ± 104.1 mm, $N = 38$) and centipedes (mean SVL = 268 ± 61.6 mm, $N = 15$). Prey class was independent of mountain range ($G = 8.24$, df = 6, $P = 0.221$), sex ($G = 3.35$, $df = 3$, $P = 0.340$) and season ($G = 3.13$, $df = 3$, $P = 0.372$). Figure 5 presents distribution of prey classes among mountain ranges and between the sexes.

I captured five species of rodents: *Peromyscus boylii*, *Neotoma mexicana*, *Eutamias dorsalis*, *Perognathus flavus* and *Sigmodon ochrognathus* (Table 8). Composition of trapped small mammals was dependent on habitat ($G = 70.82$, $df = 6$, $P \ll 0.001$), but did not vary significantly between encinal and talus habitats ($G \ll 0.01$). Trap success was highest in talus (19.8%), followed by pine-oak (15.8%), encinal (13.8%) and plateau (7.8%). Rodent diversity was highest on the plateau (Table 8). *Peromyscus boylii* comprised 64% of total captures and was most abundant in pine-oak woodland.

DISCUSSION

Diet of *Crotalus willardi* has been described only via scattered anecdotal observations largely restricted to subspecies other than *C. w. obscurus* (Table 9).

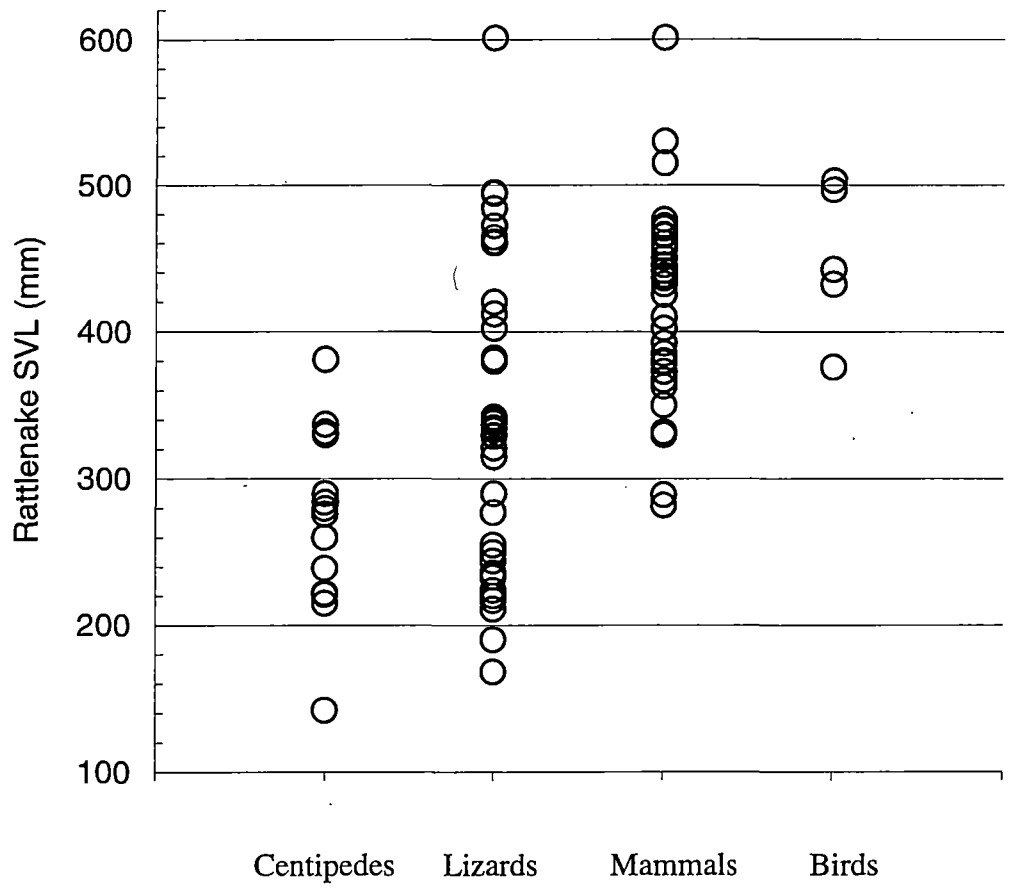

FIG. 4. Association between prey class and predator (*Crotalus willardi obscurus*) snout-vent length (N = 95).

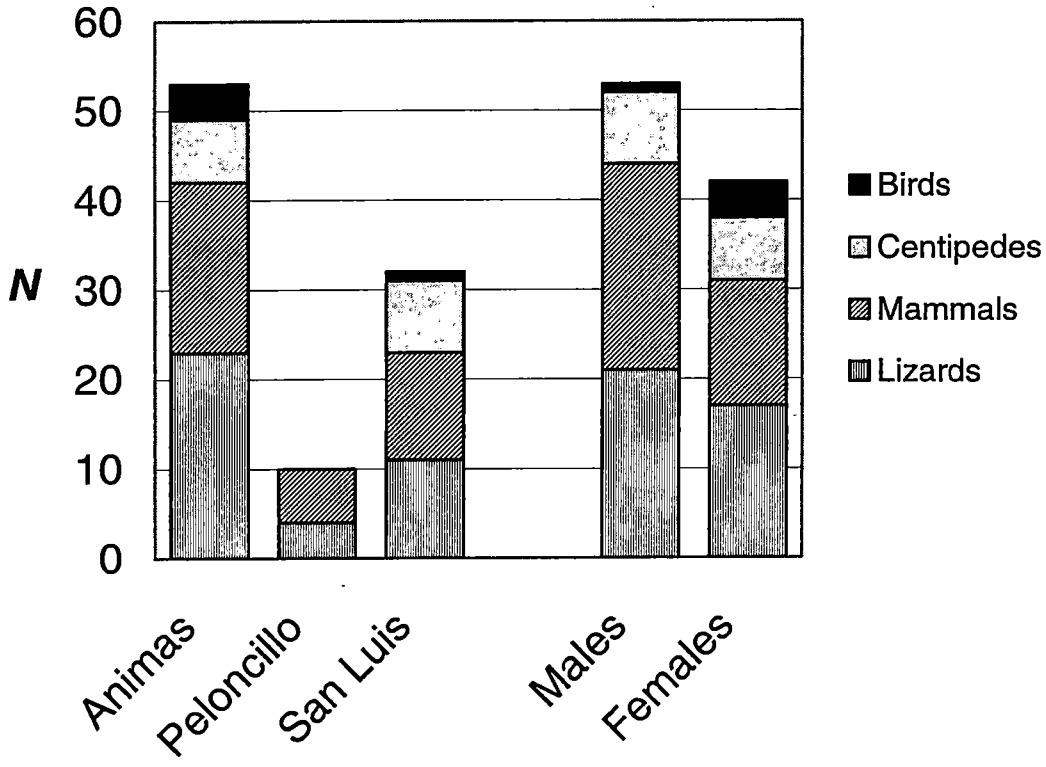

FIG. 5. Distribution of *C. w. obscurus* prey classes among three mountain populations and between the sexes.

TABLE 8. Proportion of small mammals trapped in four high elevation habitats on Animas Mountain in July and August 1996. Parentheses indicate number of individuals captured. Miscellaneous captures include *Eutamias dorsalis* ($N = 5$), *Perognathus flavus* ($N = 2$) and *Sigmodon ochrognathus* ($N = 1$)

Habitat	% *Peromyscus boylii*	% *Neotoma mexicana*	% Miscellaneous
Plateau	19.4 (6)	54.8 (17)	25.8 (8)
Encinal	61.8 (34)	38.2 (21)	0.0 (0)
Talus	62.0 (49)	38.0 (30)	0.0 (0)
Pine-oak	90.5 (57)	9.5 (6)	0.0 (0)
Pooled	64.0 (146)	32.5 (74)	3.5 (8)

TABLE 9. Summary of original prey records for *Crotalus willardi* (exclusive of *C. w. obscurus*). An asterisk (*) denotes prey consumed in captivity.

Prey	Reference
Arthropoda	
"Centipede"	Fowlie, 1965; Lowe et al., 1986*; USNM 46326
"Scorpion"	A. E. Ball, *pers. comm.* in Klauber, 1972
Aves	
Wilsonia pusilla	Marshall, 1957
Aimophila ruficeps	Parker and Stotz, 1977
Mammalia	Van Denburgh, 1922; Armstrong and Murphy, 1979; Klauber, 1949, 1972
Domestic mice*	Kauffeld, 1943; Bogert and Degenhardt, 1961; Martin, 1975a, 1976; Tryon, 1978
Peromyscus spp.	Martin, 1975c
Peromyscus boylii	Woodin, 1953
Thomomys spp.	Greene, *pers. comm.*
Squamata	
Elgaria kingii	Klauber, 1949, 1972; H. Greene, *pers. comm.*
Sceloporus jarrovii	Klauber, 1949, 1972; Woodin, 1953
Urosaurus spp.*	Martin, 1975a, 1976
*Anolis carolinensis**	Bogert and Degenhardt, 1961
*Hypsiglena torquata**	Vorhies, 1948
*Trimorphodon biscutatus**	Lowe et al., 1986

Most authors postulate that the diet of *C. willardi* consists mainly of lizards (*S. jarrovii*), but note that small mammals, centipedes and birds are also consumed (Vorhies, 1948; Manion, 1968; Harris and Simmons, 1975; Armstrong and Murphy, 1979; Lowe et al., 1986; Klauber, 1949, 1972). Several authors suggest juvenile *C. willardi* rely more heavily on lizards than do adults (Klauber, 1949,1972; Ernst, 1992; Greene, 1994). Fowlie (1965) supposed *C. willardi* might feed on *S. jarrovii*, but incorrectly speculated a "preference for amphibians and frogs, rather than warm blooded prey", based on a presumed affinity for "quasi-permanent streams." These data illustrate that while lizards represented large proportions of both juvenile (57.1%) and adult (26.4%) diets, other prey constitute significant portions as well. Noteworthy is consumption of centipedes (33.3%) by juveniles and of small mammals (62.3%) by adults. Interestingly, adults almost never ate centipedes, while only the largest juveniles consumed small mammals. In contrast to reports that *C. willardi* rarely consume birds, I found birds represent 9.4% of adult diet. While lizards are significant prey throughout life, centipedes and endotherms are clearly important at different life history stages.

Greene (1983) and Arnold (1993) predict that prey diameter/predator head size and prey/predator body mass ratios limit smaller snakes to smaller, more elongate prey. These data are consistent with this prediction, in that smaller snakes consumed only the smallest and most elongate prey taxa (centipedes, lizards). Why centipedes are essentially absent from the diet of larger snakes is unclear. Given the temporal costs of capture and digestion, ambush-hunting snakes may be under greater selective pressure to maximize prey size. When large prey are abundant, as *P. boylii* at the Animas site, adult snakes may ignore smaller prey to maximize energetic gains per unit time spent foraging. Greater

vulnerability to desiccation and/or predation may result in a more fossorial existence for juveniles, where *Scolopendra* may be more abundant and/or catchable. Conversely, adults may occupy microhabitats where *Scolopendra* are less common. Or perhaps larger snakes have difficulty manipulating small elongate objects using ingestion mechanisms designed to maximize prey size.

Although caudal luring has not been formally reported in *C. willardi* an observation has been cited as a personal communication by Schuett et al. (1984), Greene (1992) and Strimple (1992). *Crotalus w. willardi* and *C. w. obscurus* occasionally have yellow or cream-colored tails at birth (*C. w. willardi*: Martin, 1975a, 1975b; *C. w. obscurus*: Holycross, 2000) and neonatal *C. w. obscurus* often have yellowish pigment on the upper and lower labials, rostral and mental scales (Martin 1976; Holycross, 2000). Most juvenile pitvipers with brightly colored tails use the tail to lure prey (Greene, 1992). Often an ontogenetic shift in tail coloration and caudal luring behavior is associated with an ontogenetic shift in diet, from frogs or lizards as juveniles to rodents as adults (Heatwole and Davison, 1976). Thus, ontogenetic shift in tail coloration (and possibly caudal luring behavior) in *C. willardi* may be associated with an ontogenetic shift in diet. However, not all juvenile *C. w. obscurus* possess yellow tails and those that do lose this pigmentation in their first year, prior to the shift in diet reported herein.

Venom ontogeny may parallel changes in diet. Adult *C. w. obscurus* venom (LD50 = 6.4 μg/g) was approximately 33% less toxic than juvenile venom (LD50 = 4.3 μg/g) using an inbred strain (NSA) of mice (S. Mackessy, *pers. comm.*). Preliminary toxicity assays with lizards (*Uta stansburiana*) indicate that juvenile venoms kill lizards significantly faster than adult venoms (S. Mackessy, *pers. comm.*). Greater toxicity of

juvenile venom may result in functional equivalency of the venoms (as observed for *Crotalus viridis*; Mackessy, 1988), due to the significantly smaller quantities of venom produced by juveniles. Additionally, because the range of potential prey is smaller for juvenile than for adult *C. w. obscurus* (due to size constraints), higher toxicity of venom ensures that prey are rapidly immobilized and retained once envenomated. Based on preliminary results with *U. stansburiana* and on diet analysis, I predict that ontogenetic venom toxicity differential will be even greater for lizards than that observed for mice (similar to *Bothrops jararaca*; Andrade and Abe, 1999) and further tests are in progress.

These data illustrate that adult *C. w. obscurus* prey frequently on *Peromyscus* spp. Though only four records were positively identified as *P. boylii*, I expect most unidentified small mammals and all *Peromyscus* spp. samples are *P. boylii* for several reasons. Limited trapping results and surveys by Cook (1986) suggest *P. boylii* is the only *Peromyscus* syntopic with *C. w. obscurus* in the Animas Mountains. Rodent trapping results indicate that *P. boylii* is the most abundant (trappable) small mammal across habitats (64%), representing 90.5% of captures within pine-oak woodland and 62.0% within talus; the two habitats most heavily utilized by radio-tagged *C. w. obscurus*. *Peromyscus boylii* is terrestrial and arboreal (Holbrook, 1979; Cook, 1986) and relies heavily on acorns, juniper fruit and mistletoe (Jameson, 1952; Smartt, 1978). Radio-tagged *C. w. obscurus* regularly assumed classic hunting postures (*sensu* Reinert et al., 1984) at the base of partially felled trees and along fallen branches/logs used by *P. boylii* as runways into the canopy or along the ground. An observation of predation on *Peromyscus* spp. in the wild (Martin, 1975c) suggests *C. willardi* prey on small mammals

by strike-and-release, followed by strike-induced chemosensory searching (SICS; Chiszar et al., 1977).

Similarly, I expect most *Sceloporus* records from the Animas Mountains are assignable to syntopic and abundant *S. jarrovii*. *Sceloporus virgatus* occurs at lower densities and in more open habitats where *C. w. obscurus* was infrequently encountered. *Sceloporus clarkii* occurs below the lower elevational records for *C. w. obscurus* in this mountain range. In other ranges *C. w. obscurus* occurs at lower elevations and is syntopic with *S. virgatus*, *S. clarkii* and *S. grammicus*. In the Peloncillo Mountains and lower elevations of the Sierra San Luis, where *S. jarrovii* is less abundant, *C. w. obscurus* may rely more on these and other lizard species, as suggested by the eight *Cnemidophorus* spp. records obtained from these sites. Both *C. exsanguis* and *C. sonorae* are syntopic with *C. w. obscurus* and may be represented among fecal samples referred to this genus. It is unclear whether *C. willardi* strike-and-release or retain lizards; limited field observations of congeners in similar ecological settings suggest both methods may be employed. The microhabitats in which *C. willardi* adopt ambush postures for lizards and the postures themselves, differ from those used for rodents. Several individuals, particularly juveniles, were observed in loose S-shaped postures during the day against sides of rocks or in vertical fissures. The head was directed toward the top of the rock. Since this is a preferred habitat for *S. jarrovii* and *P. boylii* is largely nocturnal, I assume these individuals were ambush hunting for lizards (see Downes and Shine, 1998). Interestingly, although *Elgaria kingii* have been identified from the stomachs of *C. w. silus* and *C. w. willardi* (Table 9), this syntopic and moderately abundant lizard was

absent from this sample. *Elgaria kingii* appear to rely heavily on chemosensory stimuli and may avoid rattlesnake odors.

The high number of solitary centipede records, a skewed distribution among snake size classes and observations in captivity all suggest direct foraging on live centipedes rather than secondary ingestion or incidental scavenging of dead ones. Indeed, the first *C. willardi* collected and later deposited in a museum (USNM 46326; Nelson Goldman Expedition, Colonia Garcia, Chihuahua, 3 July 1899) is a small *C. w. silus* that contains a large, intact *Scolopendra* spp., swallowed headfirst. Large centipedes are not uncommon in the diet of several other rattlesnakes (e.g., *Sistrurus miliarus*, Hamilton and Pollack, 1955; *Crotalus enyo*, E. Taylor, *pers. comm.*; *Crotalus lepidus klauberi*, ATH, *unpubl. data*; *Sistrurus catenatus edwardsii*, ATH, *unpubl. data*). Centipede eaters may have evolved specific adaptations for foraging on venomous prey. Rubio (1998) wondered if centipede eaters are somewhat resistant to centipede venom and two observations suggest centipede-specific prey handling behaviors. *Crotalus w. willardi* preying on centipedes in captivity raise the anterior half of the body above the prey, strike down and release, and then rapidly retreat (H. McCrystal, *pers. comm.*). On the other hand, Rubio (1998) described a predatory sequence by a captive *C. lepidus* wherein a centipede was struck just behind the head and was not released prior to ingestion. I was unable to determine direction of ingestion in this study because all of the centipede records were identified from fecal remains, however centipedes found in the stomachs of *S. catenatus edwardsii* (and USNM 46326 cited above) were all swallowed headfirst, judging by orientation in the stomach (ATH, *unpubl. data*).

A single field observation suggests *C. willardi* strikes and restrains birds using the mouth and body (Parker and Stotz, 1977). Pitvipers rely on SICS to follow the trail of envenomated terrestrial prey, a foraging strategy incompatible with flying prey. It is unclear what cues rattlesnakes use to determine predatory response. However, the observation of arboreal foraging on birds (Parker and Stotz, 1977) and my recurrent observations of radio-tagged individuals perched in trees and low bushes (see also Rossi and Feldner, 1993), suggest *C. w. obscurus* may adopt foraging postures specifically for birds, perhaps in response to chemical cues on frequently used perches.

Woodlands and adjacent talus slopes and rock outcrops play a central role in *C. w. obscurus* foraging ecology. *Peromyscus boylii* is most abundant in these habitats (Table 8) and adult snakes exhibit foraging tactics that use structural components of woodland. Likewise, talus slopes and rock outcrops near woodlands support the highest densities of *S. jarrovii*. *Scolopendra spp.* are probably more abundant in forest leaf litter and in talus than on hot exposed grassy slopes. Identified avian prey species are associated with woodlands and chaparral. At the Animas site, *C. w. obscurus* were infrequently encountered in immediately adjacent open habitats where encounters with humans were much more likely; they also occur in much lower densities in mountain ranges where pine-oak communities are less developed, such as the Peloncillo Mountains. *Crotalus w. obscurus* foraging ecology is predominately adapted to woodland and talus communities and may play a role in habitat selection. Clearing of woodlands, by mechanical means or by stand-replacing, natural or management-ignited catastrophic fire, would undoubtedly negatively affect *C. w. obscurus* prey communities and structural components of habitat

used in foraging. Preservation of encinal and pine-oak woodlands and associated faunal communities is essential to conservation of this federally threatened rattlesnake.

Montane Rattlesnakes and Prescribed Fire

Historically fire was a common and natural occurrence in Madrean Province ecosystems of northern México and southwestern United States and a significant factor in ecosystem maintenance (Leopold, 1924; Marshall, 1957; Swetnam and Baisan, 1994, 1996). Pre-Euroamerican fire frequency is well understood in high elevation pine-dominated communities through dendrochronological analyses (e.g., Swetnam and Baisan, 1994, 1996). In lower elevation encinal woodlands (*sensu* Lowe, 1967), a lack of data prevents clear understanding of prehistoric fire (Kaib et al., 1996; Kruse et al., 1996). However, fire return intervals of four (within canyons) to eight years (synchronous among canyons) in adjoining pine-oak dominated canyons suggest encinal woodlands burned frequently (Kaib et al., 1996). Regardless of past history, fire frequency in these communities has decreased greatly in modern time as a result of American settlement patterns, active fire suppression, and reduction of fine fuels by livestock grazing (Bahre, 1991; Caprio and Zwolinski, 1994; Kruse et al., 1996; Swetnam and Baisan, 1996). Altering and reducing the role of fire has resulted in dense stands, accumulated fuels in woodlands, and altered species composition (Marshall, 1957; McPherson et al., 1993; Kruse et al., 1996; Swetnam and Baisan, 1996). After nearly a century of suppression, fuel loads often are greatly elevated above natural levels, increasing potential for catastrophic fire (Marshall, 1957; Caprio and Zwolinski, 1994; Kaib et al., 1996; Swetnam and Baisan, 1996). In this context, prescribed fire might be detrimental rather than therapeutic to ecosystem health.

Fire can have a significant effect on reptile populations (Lillywhite and North, 1974; Mushinsky, 1985; Braithwaite, 1987; Pianka, 1989; Mushinsky, 1992;

Friend, 1993). However, data depicting response of reptiles to fire are limited and too varied to permit generalizations (Bamford 1985; Quinn, 1994). Effects may be beneficial, detrimental, or benign to reptile populations. Although fire might result in direct mortality (e.g., Erwin and Stasiak, 1979), it also might improve habitat and therefore facilitate population growth or stability (Mushinsky, 1985; Greenberg et al., 1994; Curtin, 1997). To further confound matters, different fire intensities and frequencies often have dissimilar effects (e.g., Braithwaite, 1987; Pianka, 1989; Mushinsky, 1992). Assessment of fire effects on reptile populations is complex, and requires a species- and habitat-specific approach. A prevalent assumption is that fire in unperturbed systems is beneficial to constituent fauna, or at worst, benign. However, fire in perturbed systems, or prescribed fire at unnaturally high frequencies, may have dramatically different effects on reptile populations than natural fire in unperturbed systems.

In this field study, I describe behavior of montane rattlesnakes through a prescribed fire in Madrean woodlands and savannas. Although previous studies were forced by circumstance to concentrate on post-fire effects (e.g., Erwin and Stasiak, 1979; Mushinsky, 1985; Braithwaite, 1987; Simons, 1979; Mushinsky, 1992; Greenberg et al., 1994; Whelan, 1995), I was able to evaluate snake behavior before, during, and after fire. I paid particular attention to *Crotalus willardi obscurus*, a federally threatened montane rattlesnake (U.S. Fish and Wildlife Service, 1978).

Methods and Materials

In June 1997 the Maverick Prescribed Burn was ignited in the southern Peloncillo Mountains (Coronado National Forest) of extreme southern Arizona and New Mexico. The objective of the fire was to create a mosaic of burned and unburned fuels with 35%

to 60% burned, in order to increase forage production and maintain biodiversity (United States Forest Service, 1997). The fire was limited to a 6,885 ha primary perimeter south of Forest Road 63 (Geronimo Trail), north of Sycamore Creek, and west of Maverick Spring. A 56,700 ha secondary perimeter outlined the maximum boundary of the project. Delayed Aerial Ignition Devices, Alumagel, and drip torches effected ignition. The burn area spanned 1280–1920 m elevation and consisted of a mosaic of plant associations (desert grassland, desert scrub, encinal woodland, oak and pinyon-juniper savannah, and patches of pine-oak woodland in canyon bottoms). Observations herein are confined to high elevation communities dominated by oak (*Quercus*), alligator bark juniper (*Juniperus deppeana*), Mexican pinyon (*Pinus cembroides*), Chihuahua pine (*Pinus leiophylla*), and perennial grass.

Although 6 rattlesnake species occur within the primary perimeter of the fire, only Blacktail (*Crotalus molossus*), Rock (*C. lepidus*), and New Mexico Ridgenose (*C. w. obscurus*) rattlesnakes occupy the high elevation sites where I focused my observations. The Peloncillo Mountains population of *C. w. obscurus* is one of three documented montane isolates. *Crotalus w. obscurus* is exceptionally rare in the Peloncillo Mountains where each capture requires an average of 33 person-days (264 h) of dedicated searching (A. T. Holycross, pers. obser.). Prior to this study, 11 specimens had been documented in the Peloncillo Mountains, all within the secondary fire perimeter and eight of these within the primary perimeter.

I performed radio-telemetry between 5 May and 30 July 1997 within the primary perimeter at two locations ca. 3 km apart. The north site (1890 m) was located on a high plateau and bordering escarpment. I tracked one male *C. molossus* (CM1) and four male

C. lepidus (CL1, CL2, CL3, CL5) at the north site. The south site (1860 m) was located in the headwater drainage of an eastern canyon. I tracked two males (CW1, CW2) and one pregnant female (CW3) *C. w. obscurus* and one male *C. lepidus* (CL4) at the south site. I selected individuals for implantation based on mass and sex. Sex was determined by probing for hemipenes (Schaefer, 1934) and reproductive status of females by palpation (Fitch, 1960). When possible, males were selected over females to avoid confounding effects related to female reproductive state. Transmitters (Holohil Inc., Carp, Ontario), weighing between 1.3 g and 2.8 g, and with an average transmitting distance of 25 m, were surgically implanted in the body cavity (Reinert and Cundall, 1982). Isoflourane was used as an anesthetic.

Radio-tagged individuals were released at point of capture and located once every day, resulting in 483 observations. Observations were divided into three periods: A) initiation of telemetry to time fire affected each snake, B) post-fire to onset of summer rainy season (17 July 1997), and C) onset of summer rainy season to end of study (30 July 1997). Onset of the rainy season was estimated from field data on precipitation and cloud cover, and data from a weather station in Douglas, Arizona (http://www.ncdc.noaa.gov).

Observations were brief (<10 min) to minimize disturbance. Location was recorded using a handheld Global Positioning System (GPS) receiver (Trimble Geo Explorer II). Data were stored as GPS files and mapped from differentially corrected Universal Transverse Mercator (UTM) coordinates. Activity areas (convex polygon) were calculated for each period using McPAAL software (Stuwe and Blohowiak, 1992). Activity and movement measures were calculated for each of the three periods of

observation. Pair-wise comparisons of these measures included all individuals for which data were available in both periods under consideration (Table 1). Reported values were calculated as follows: daily activity area = activity area divided by the number of days observed, movement rate = total distance moved divided by number of days observed, and movement magnitude = total distance moved divided by number of movements (>2 m). Movement frequency was number of days an individual moved (>2 m) divided by number of days observed. Each snake's microhabitat was categorized as subterranean (deep within a refuge, usually not visible), cavity (in rock/earth near the surface, <50% of body exposed), plant (beneath plant leaves, bark, etc., <50% of body exposed), or surface (>50% of body exposed).

RESULTS

The north site burned 24–25 June, with fire originating in a basin, sweeping up steep slopes, and crossing the plateau. Fire was particularly intense (flame lengths > 40 m) on wooded slopes where high fuel load and prevailing winds enhanced pre-heating and convection. Here, large tracts of trees and smaller shrubs, yucca, agave, and cacti were incinerated and surface rocks fractured by heat. On the plateau, where recent grazing and previous fires reduced fuel load, a less-intense ground fire left some areas unburned. Four snakes (CM1, CL1, CL2, and CL3) weathered the fire on the plateau, and one (CL5) was on the adjacent wooded slope.

At the south site, sweeping ground fire on 27 June was of lower intensity and resulted in a mosaic of burned and unburned areas. There was little damage to large trees and agave, with few exceptions. In burned areas, cacti and yucca were scorched, grasses and forbs burned to ash, and rocks blackened.

All snakes were located <18 h pre-fire, and <24 h post-fire. Fire passed directly over eight of these, and within 3 meters of the ninth (CW1). A male *C. lepidus* (CL5, Arizona State University #31334) was found dead within a crevice on a rock face on 26 July, immediately post-fire. Though desiccated, the body showed no signs of scorching. All adjacent understory and shrub vegetation had been burned to ash. Nearby trees were incinerated, thick ash covered the ground, and rocks were fractured. To reach this crevice, CL5 moved 60 m from its pre-fire location. The remaining eight individuals survived the fire and were found in subterranean retreats. Two of these (CW3 and CM1) had moved since they were located before the fire.

Two other dead vertebrates were found: burning wood rat (*Neotoma*) during the fire, and on 30 June a slightly burned Sonoran whipsnake (*Masticophis bilineatus*, ASU #31335) on leaf litter approximately 30 m from the perimeter of a burned area. Other dead fauna included numerous cicadas (*Platypedia*) and other small invertebrates. A striped plateau lizard (*Sceloporus virgatus*) foraged on dead insects among embers and ash during the fire.

TABLE 10. Summary data on activity measures for radio-tagged rattlesnakes. Letters indicate period of observation: A = pre-fire dry season, B = post-fire dry season, and C = post-fire rainy season. Daily activity area = activity area/day, movement rate = distance moved/day, movement magnitude = distance moved/movement, movement frequency = days moved/days observed. Standard errors are presented in parentheses. Asterisks indicate no data are available; snake CL4 could not be located during period C and the fire killed CL5.

Snake	Number of observations			Daily activity area (m^2)			Movement rate (m)			Movement magnitude (m)			Movement frequency (%)		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
CW1	43	18	11	19.5	0.4	58.3	2.3 (0.7)	0.6 (0.4)	8.7 (3.5)	5.4 (1.5)	5.5 (2.5)	10.7 (4.0)	41.5	11.1	81.8
CW2	40	18	12	74.4	109.7	223.4	8.0 (2.0)	8.2 (2.1)	17.4 (7.2)	14.7 (3.1)	12.3 (2.4)	26.1 (9.4)	53.7	66.7	66.7
CW3	16	18	12	5.6	0.0	1.3	1.3 (0.9)	0.0	0.9 (0.7)	6.3 (3.2)	0.0	3.6 (2.2)	21.4	0.0	25.0
CL1	26	14	5	38.6	489.5	1454.3	4.7 (1.3)	29.1 (9.4)	24.5 (20.4)	8.5 (1.8)	40.7 (11.2)	49.0 (36.0)	55.6	71.4	50.0
CL2	28	6	3	591.8	1.3	37.3	23.8 (3.1)	3.8 (2.5)	21.5 (3.5)	24.7 (3.1)	11.5 (1.5)	21.5 (3.5)	96.3	33.3	66.7
CL3	16	20	13	11.6	66.1	954.6	3.1 (2.0)	4.3 (2.2)	26.0 (8.5)	9.3 (5.4)	17.0 (6.0)	48.3 (9.6)	33.3	27.8	53.8
CL4	24	11	**	139.9	22.2	**	10.9 (3.4)	2.4 (6.3)	**	15.4 (4.4)	13.8 (2.2)	**	71.4	40.0	**
CL5	51	**	**	101.4	**	**	7.4 (2.1)	**	**	13.4 (3.3)	**	**	92.9	**	**
CM1	45	20	13	715.6	159.4	94.4	12.6 (3.4)	9.5 (5.8)	7.9 (2.4)	20.8 (5.1)	23.8 (13.3)	14.6 (2.2)	60.4	29.4	53.9

Individual snakes generally remained within burned areas during period B (75.6% of locations). Pooled daily activity areas (Table 1) did not significantly differ ($P > 0.05$) in pair-wise comparison of periods (Wilcoxon's Signed-Rank Test; $N = 8$ for A–B; $N = 7$ for B–C and A–C). Five individuals (CW1, CW3, CL2, CL4, CM1) had smaller daily activity areas in period B than in period A. Three (CW2, CL1, CL3) exhibited the reverse trend. Daily activity areas were larger in period C than period B for 6 individuals (CW3, CW1, CW2, CL1, CL2, and CL3), and less for one (CM1) that had recently fed. I was unable to locate CL4 throughout period C. The smallest daily activity areas were exhibited by *C. w. obscurus* (all periods combined: CW1 = 22.3 m^2; CW2 = 82.4 m^2; CW3 = 4.1 m^2). Neither rate nor magnitude of movements (Table 1) differed significantly ($P > 0.05$) in pair-wise comparisons between periods (Wilcoxon's Signed-Rank Test; $N = 8$ for A–B; $N = 7$ for B–C and A–C). Nevertheless, most individuals moved shorter than average distances for several days post-fire. All individuals moved less frequently in period B when compared to either period A (Wilcoxon's Signed-Rank Test, $N = 8$, $P = 0.04$) or C ($N = 7$, $P = 0.04$). Movement frequency between A and C did not significantly differ ($N = 7$, $P > 0.05$).

Microhabitat use differed significantly among periods (RxC test of independence, $G = 67.05$, $df = 6$, $P << 0.01$). Figure 6 illustrates a marked increase in use of subterranean refugia and a decrease in number of snakes found on the surface after fire (period B). This trend reversed with the arrival of the monsoon (period C) and the beginnings of herbaceous recovery. Snakes were observed crawling 5% and 12% of observations in period A and C respectively, but were never found crawling during period B.

I assessed fire effects on *C. w. obscurus* habitat by revisiting three collection localities that burned in this fire. Repeated fires over the last two decades have affected one site (the south site for the radio-telemetric portion of this study). Consequently, fire in this area was of low intensity and removed what fuels had accumulated, but did not remove woodland components. In contrast, two collection sites (in Whitmire and Cottonwood canyons) with no recent burn history burned intensely, resulting in complete removal of woodland components, extensive erosion on slopes harboring rattlesnake refugia, and severe sedimentation in streambeds.

Discussion

These observations suggest that high intensity fires may result in higher mortality of montane rattlesnakes than do low intensity fires, although definitive conclusion is prevented by small sample size. Nevertheless, the single individual exposed to conflagration died; eight others exposed to low intensity ground fire survived. Observations of snakes surviving or dying in fires are not unique to this study. Simons (1989) found four dead snakes, after a chaparral fire in Arizona. Dead and fire-injured *Pituophis melanoleucus* and *Thamnophis* are often observed after prescribed fires in tallgrass prairies (Erwin and Stasiak, 1979). Two radio-tracked *Elaphe obsoleta* survived a prescribed fire in a shortleaf pine (*Pinus echinata*) forest (Withgott and Amlaner, 1996). David Barker (*pers. comm.*) reported two (of three) radio-tagged *C. w. obscurus* survived an exceptionally intense fire in the Sierra San Luis in 1989. The third specimen

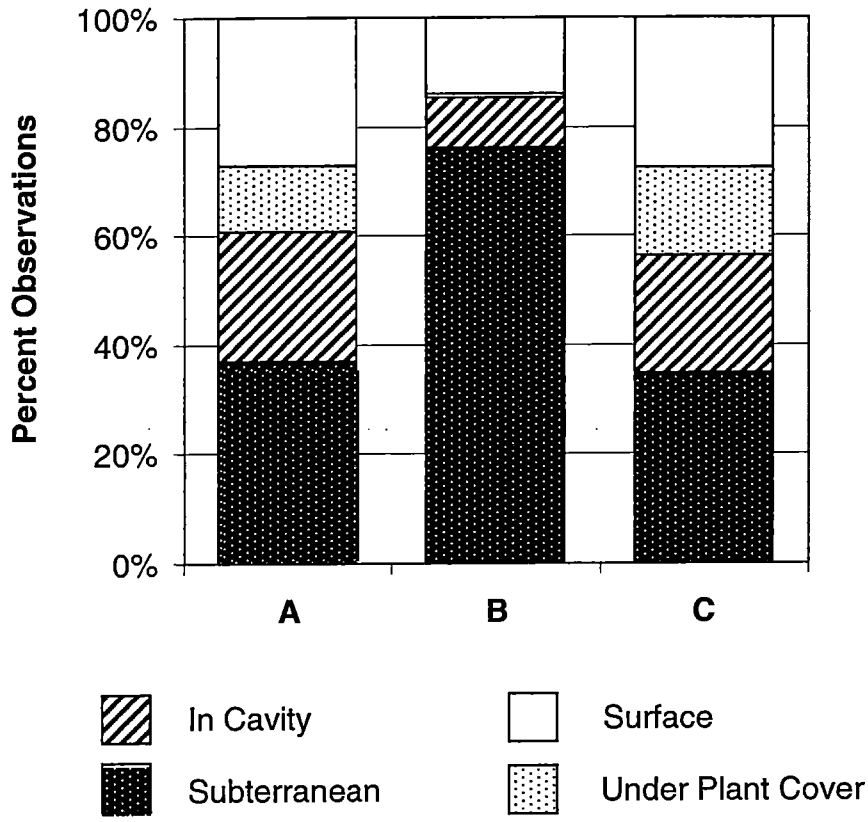

FIG. 6. Microhabitat use by nine montane rattlesnakes through a prescribed fire. Period A = pre-fire dry season, period B = post-fire dry season, and period C = post-fire rainy season (N = 296, 122, and 69 observations respectively).

was not found after the fire. Barker also observed a reduction in capture rate, fire-injured individuals, decimation of woodlands, and severe erosion. All nine snakes in the present study were in various refugia during the fire, including the individual that died. This may not constitute active avoidance of fire but may instead be coincident with the observation that reptiles in arid climates normally occupy (or at least are near) insulating refugia. Mortality often is inferred from post-fire presence of dead snakes or a decrease in capture rate. This study suggests rattlesnakes often weather fire underground and may die within retreats where it is unlikely they would be found. Similarly, decrease in capture rate may be due to increased use of subterranean refugia in burned landscapes. Thus, conclusions about effects of fire on snake mortality are best derived from mark-recapture data or from large samples of radio-tagged individuals.

The inclusion of a control group in an unburned plot would allow a direct test of fire effects on rattlesnake behavior. Unfortunately, although such a test is desirable, it is not logistically feasible given the rarity of montane rattlesnakes in this range, their remote and rugged habitats, and the unpredictability of fire. Although spatial descriptors of activity did not differ significantly between periods A and B, snakes moved significantly less frequently in period B. Snakes also were found deeper in refugia (subterranean) during period B when compared to period A, and were never found crawling in period B. Absence of observed diurnal movement in period B and no difference in spatial descriptors of movement between periods, suggests the possibility of a shift to crepuscular or nocturnal movements. Removal of ground cover may retard diurnal movements by increasing surface temperatures or by increasing visibility to predators. These provide adaptive explanations for an increase in fossorial behavior, a shift to

nocturnal movement, and a decrease in frequency of movement in a burned landscape. In most years, seasonal activity of montane rattlesnakes is characterized by a period of surface activity during the early summer (late May to early July) that may increase following the advent of summer rains in mid-July (Armstrong and Murphy, 1979; Lowe et al., 1986; Ernst, 1992). When reasonably considered in light of the natural history of these species, these data intimate that rattlesnake behavior is altered in response to fire-induced changes in the environment. If real, these shifts in behavior may manifest differences in foraging success, energy budgets, predation, or reproduction, and could ultimately influence demographic parameters.

Long-term effects of various prescribed fire regimes on population dynamics of *C. w. obscurus* are difficult to assess. Chronic changes in habitat or prey populations (e.g., Bock and Bock, 1978; Bigalke and Willan, 1984; Quinn, 1994; Whelan, 1995) may ultimately shift population demographics, densities, or distributions of rattlesnakes. As an element of the Pine-Oak Woodland herpetofaunal assemblage, *C. willardi* is "predominantly adapted to montane pine-oak woodlands" of the Sierra Madre Occidental" (McCranie and Wilson, 1987:18). In the Peloncillo Mountains, as in adjacent ranges, *C. w. obscurus* is most often encountered in wooded canyon bottoms and on steep wooded slopes (Degenhardt et al., 1996). Thus, *C. w. obscurus* undoubtedly evolved and persists in open woodland communities maintained by fire. Fire suppression has altered elements of these communities considerably. These observations suggest increased fuel loads in wooded canyons and on wooded slopes result in destruction of *C. w. obscurus* habitat (encinal and pine-oak woodlands) previously maintained by frequent, low intensity fire. *Crotalus w. obscurus* is exceptionally scarce in the Peloncillo Mountains,

with habitat naturally fragmented into canyon isolates. As a consequence, this mountain range may support a metapopulation of *C. w. obscurus*, with low levels of interdemic migration and gene flow. If isolated demes are extirpated, recolonization from adjacent canyons might take decades, even if vegetative recovery is rapid. Thus, the fate of each habitat patch may be critical to conservation of this population. Consistent misapplication of prescribed fire might ultimately lead to extirpation of species like *C. w. obscurus* that are dependent on woodland habitats (McCranie and Wilson, 1987). On the other hand, inability to restore fire as a natural component of the system will result in further accumulation of fuels, consequently increasing risk of catastrophic fire. Both restoring the natural role of landscape-level summer fire and preservation of encinal and pine-oak woodlands are critical to conservation of this subspecies. In the Peloncillo Mountains, both objectives can be achieved through fuel reductions in many woodland habitats. Until fuel loads are reduced, summer fire (prescribed or naturally ignited) poses a significant threat to habitat for *C. w. obscurus*.

Microsatellite variability in populations of *Sistrurus catenatus edwardsii* from Arizona and New Mexico.

Data from nuclear DNA markers can illuminate patterns of genetic variation among and within populations and thus provide critical insights to demographic structure. Levels of genetic variability within isolated populations is largely a consequence of population size (extant and historical) and degree of isolation, but is also theoretically tied to future viability. Where population sizes are small (or have bottlenecked in the past) and/or levels of gene flow are negligible, populations are susceptible to loss of genetic variability via inbreeding or genetic drift and are theoretically at increased risk of extinction. The use of nuclear DNA markers, particularly microsatellites, for assessing these patterns in a wide variety of taxa has exploded in recent years due to the relative efficacy of lab procedures. However, snakes have been underrepresented in such evaluations, due primarily to their secretive nature and the concomitant difficulty of acquiring suitable sample sizes for analysis. Relatively few studies have evaluated nuclear DNA variation in snakes (Gibbs et al., 1994; Gibbs et al., 1997; Bushar et al., 1998; Prior et al., 1997; Lougheed et al., 1999; Prosser et al., 1999).

The Desert Massasauga (*Sistrurus catenatus edwardsii*) is found from western Texas across southern New Mexico and into extreme southeastern Arizona in the U. S. A., with outlying populations in southeastern Colorado and south Texas, U. S. A. and in Coahuila and Nuevo Léon, México. Throughout its range *S. c. edwardsii* consists wholly of isolated populations, many of which are declining, extirpated, or perceived to be at risk of decline (Lowe et al., 1986; Greene, 1997; Werler and Dixon, 2000). This patchy distribution appears to be a consequence of both narrow ecological tolerances and Holocene climate changes that have fragmented suitable habitat (Greene, 1997).

More recently anthropogenic desertification, encroachment by agriculture, road mortality, and willful extermination have been identified as threats or causative agents in the decline and extirpation of populations (Lowe et al., 1986; Greene, 1997; Hammerson, 1999; Werler and Dixon, 2000). Management of relict populations of *S. c. edwardsii* would benefit from an understanding of patterns of genetic variability among and within populations. This information can be used to define local populations, elucidate population substructure, and determine levels of inbreeding. With such information managers are better equipped to define management units (*sensu* Moritz, 1994), prioritize populations for conservation, and rank populations with regard to their conservation value.

Herein, I quantify genetic variability in two populations of *S. c. edwardsii* (in Arizona and New Mexico) using microsatellite DNA loci isolated from this species (Gibbs et al. 1998) and successfully employed toward similar ends with eastern populations (Gibbs et al. 1997). Microsatellites are tandem repeats of short sequences of nucleotides that are hypervariable in length (number of sequence repeats), each length polymorphism comprising a distinct allele for the locus (Tautz, 1989; Quellar *et al.*, 1993). Microsatellite loci are distributed throughout the genome, selectively neutral, co-dominant, inherited in a simple Mendelian fashion, and assignable to specific loci (Quellar *et al.*, 1993; Ashley and Dow, 1994). This combination of attributes makes them an ideal genetic marker for studies of kinship, paternity, inbreeding, and fine-scale population structure. In addition, the comparatively rapid mutation rate of microsatellite alleles may more accurately reflect the evolution of additive quantitative genetic variation in small populations, which occurs at similarly high rates.

METHODS AND MATERIALS

Populations Sampled

I sampled tissue from *S. c. edwardsii* in the course of conducting autecological investigations in Cochise County, Arizona (1993–1997) and Valencia County, New Mexico (1997–1998). The Arizona population is ca. 410 km southwest of the New Mexico population (Fig. 7). I sampled blood from the caudal vein of live snakes and immediately stored this in either 98% ethanol or Queen's lysis buffer (Seutin et al., 1991). I individually marked live snakes using passive integrated transponders (Jemison et al., 1995) to prevent unintentional resampling of individuals. From dead snakes I excised small pieces of muscle and preserved these in Queen's lysis buffer. The majority of dead snakes were subsequently deposited in museums (Appendix E). A single sample was obtained from a shed (definitively identified as a Massasauga) found in the field. Although sampling efforts spanned April through October, in most years I emphasized late summer and fall sampling.

The Arizona population inhabits a tobosa (*Hilaria mutica*) grassland that blankets a volcanic cinder field in the San Bernardino and San Simon Valleys. This is the sole substantial population in Arizona; all other populations appear to be extirpated or approaching extirpation. Between 1993 and 1997 I collected 123 individuals (56 of which were tissue-sampled and genotyped) along a 16.9 km (10.5 mile) transect

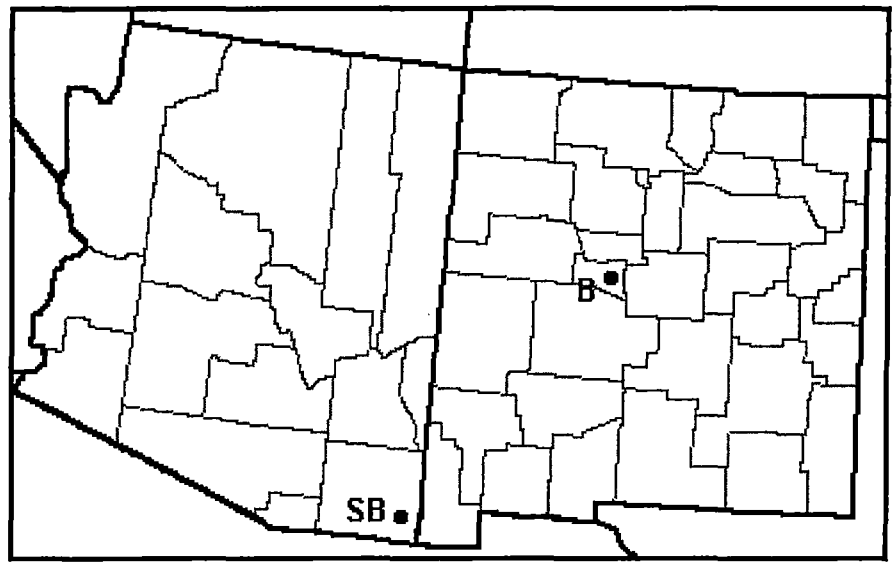

FIG. 7. Location of *Sistrurus catenatus edwardsii* populations sampled in Arizona and New Mexico. SB = San Bernardino population, B = Belen population.

(Highway 80) that bisects the grassland and Massasauga population (Fig. 8). Typically, I drove back and forth along this transect for several hours after sunset, essentially spending equal time on all portions of the roadway.

The New Mexico population occupies a shortgrass prairie in the Rio Grande Valley east of Belen and west of the Manzano Mountains. Twenty-two individuals were sampled throughout an area ca. 28 x 13 km. The vast majority of this area is under development for tract homes, and many of the specimens were sampled from roads installed to support the development.

Laboratory Methods

Procedures used to extract DNA from samples and characterize the microsatellite loci are provided in Gibbs et al. (1998). In brief, DNA was extracted from blood or muscle samples using DNAZOL (Gibco) or standard phenol-chloroform procedures and quantified using a fluorometer. Individuals were then genotyped at each of six dinucleotide microsatellite loci (Scμ 01, 05, 07, 11, 26, and 140). Based on results reported for five populations of *S. c. catenatus*, the first five of these loci had expected heterozygosities between 0.257 and 0.879 and appeared to segregate in a Mendelian fashion (Gibbs et al. 1997). Individuals were genotyped using PCR amplifications performed in 10 μl reaction volumes using 50 ng of genomic DNA, 0.3 pmole of the forward primer end-labeled with [^{33}P]–ATP (Dupont), 0.4 pmole of unlabelled forward primer, 0.8 pmole of unlabelled reverse primer, 200 μM dNTPs, 0.5 U of AmpliTaq (Perkin Elmer), 0.1 M Tris-HCl, pH 8.3, 0.5 M KCl, and 1.5–2.0 mM $MgCl_2$. Amplifications were run in a Perkin Elmer 480 Thermocycler for 30 cycles with each

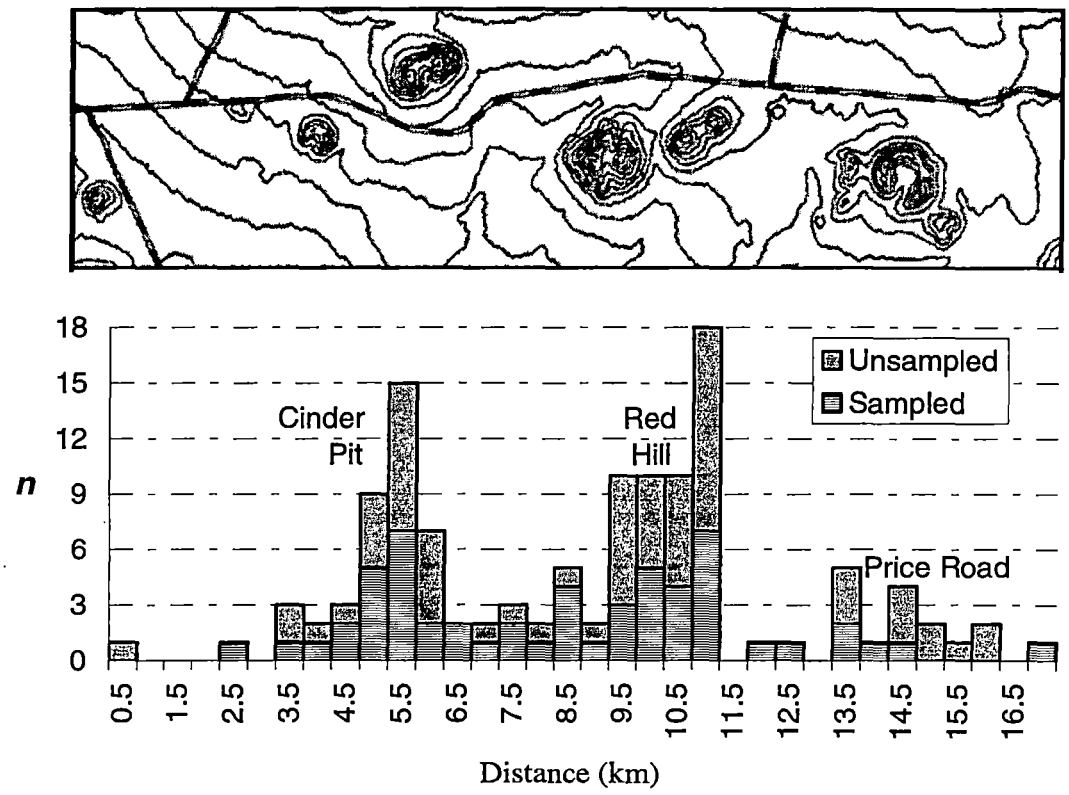

FIG. 8. Distribution of Massasauga sampled along a 17 km transect (Highway 80) in the San Bernardino Valley, Cochise County, Arizona. Specimens that were tissue-sampled and used in this study are discriminated from specimens that were not tissue-sampled.

cycle consisting of 30 s at 94°C, 30 s at the appropriate annealing temperature (described in Gibbs et al., 1998) and 30 s at 72°C. Amplification products (3 μL) were run on 6% denaturing polyacrylamide gels at 55 W for 2.5 h. Gels were dried and exposed to Biomax (Kodak) X-ray film overnight. Products were sized by reference to a known sequencing reaction of a control template and 'hot' amplifications of the known-size clones for each locus, both of which were run on the gel at the same time.

Analysis of Variation

To facilitate straightforward comparison with the results from eastern populations of this species (Gibbs et al. 1997), I used similar analytical tools and software. I quantified differentiation between the populations by testing for differences in allele frequencies and by calculating fixation and distance indices. Some loci used in this study have disjunct distributions of alleles within populations (discussed by Gibbs et al., 1998), suggesting these loci do not strictly follow the step-wise mutation model assumed by some measures of population differentiation developed for microsatellites [e.g. R_{ST} (Slatkin 1995) and dmμ (Goldstein et al. 1995)]. Therefore, I present fixation and distance measures based on the infinite alleles model (F_{ST} and Nei's D). I used several analytical software packages to calculate these measures including: GENEPOP 3.2a (Raymond and Rousett, 1995) to test for differences in allele frequencies and heterozygote deficiencies and FSTAT (Goudet 1995) to calculate and test F_{ST} values. Inititial manipulations of data and input files were created using the MS Toolkit macro for Excel.

I estimated levels of nonrandom association of alleles within Desert Massasauga populations by (*i*) testing for both locus-specific and overall heterozygote deficiencies within populations using the exact tests in GENEPOP and, (*ii*) calculating F_{IS} values for each locus and then an overall F_{IS} value using data pooled across all loci using FSTAT. The significance of the overall F_{IS} value of each population was tested using the permutation procedure in FSTAT.

RESULTS

Genetic characteristics of the six loci within each population are provided in Table 11. All six showed high levels of variability within the two populations, rendering them useful for investigations of population structure and differentiation. Generally Scμ 05, 11, and 140 were more variable than the remaining loci in terms of numbers of alleles, mean allele frequencies, and heterozygosity. However, there was considerable variability in these characteristics between populations (Table 11).

Population differentiation

Allele frequencies in the two populations significantly differed at five of the six loci ($P = 0$ for each test), but did not significantly differ at Scμ 26 ($P = 0.147$). Combined across all loci these tests were highly significant ($P \approx 0$) illustrating that allele frequencies in these populations have significantly diverged. Additionally, both populations contained a high proportion of unique alleles ranging from 0 to 70 % by locus/population (Table 12). Across all loci, 26.9 % (Belen) and 50.6 % (San Bernardino) of alleles were detected only in the source population. Furthermore, 7.7 % (Belen) and 19.0 % (San Bernardino) of alleles in these populations occurred at frequencies $\geq 5\%$. The higher

TABLE 11. Genetic characteristics of six microsatellite loci in two *Sistrurus catenatus edwardsii* populations. N = number of individuals genotyped; No. alleles = numbers of different sized alleles present in the population; H_{exp} and H_{obs} = expected and observed heterozygosities as calculated using GENEPOP. r_c and r_b are estimates of the frequency of null alleles as described by Chakraborty et al. (1992) and Brookfield (1996), respectively, $r_c = (H_{exp}–H_{obs})/(H_{exp} + H_{obs})$ and $r_b = (H_{exp}–H_{obs})/(1 + H_{exp})$.

	Population	
Locus	Belen	San Bernardino
Scμ 01		
N	20	54
No. alleles	7	10
Size range (in bp)	145–166	145–160
Mean frequency (± SD)	0.14 ± 0.24	0.10 ± 0.09
H_{exp}	0.532	0.830
H_{obs}	0.350	0.815
r_c	0.206	0.009
r_b	0.119	0.008

continued

TABLE 11, continued

Scμ 05		
N	19	54
No. alleles	10	17
Size range (in bp)	163–241	163–245
Mean frequency (± SD)	0.10 ± 0.11	0.06 ± 0.06
H_{exp}	0.804	0.900
H_{obs}	0.737	0.778
r_c	0.043	0.073
r_b	0.037	0.064
Scμ 07		
N	19	56
No. alleles	7	10
Size range (in bp)	184–202	166–200
Mean frequency (± SD)	0.14 ± 0.18	0.10 ± 0.15
H_{exp}	0.696	0.702
H_{obs}	0.789	0.696
r_c	-0.063	0.004
r_b	-0.055	0.003

continued

TABLE 11, continued

Scμ 11		
N	21	56
No. alleles	9	16
Size range (in bp)	66–126	70–134
Mean frequency (± SD)	0.11 ± 0.10	0.07 ± 0.08
H_{exp}	0.825	0.848
H_{obs}	0.905	0.857
r_c	-0.046	-0.005
r_b	-0.044	-0.005
Scμ 26		
N	22	55
No. alleles	5	11
Size range (in bp)	265–275	167–275
Mean frequency (± SD)	0.20 ± 0.19	0.09 ± 0.12
H_{exp}	0.678	0.764
H_{obs}	0.591	0.764
r_c	0.068	0.000
r_b	0.052	0.000

continued

TABLE 11, continued

Scμ 140		
N	20	55
No. alleles	13	15
Size range (in bp)	95–126	78–126
Mean frequency (± SD)	0.08 ± 0.05	0.07 ± 0.11
H_{exp}	0.913	0.758
H_{obs}	0.800	0.673
r_c	0.066	0.059
r_b	0.059	0.048

Table 12. Distribution of alleles unique to each *Sistrurus catenatus edwardsii* population. *T* is the total number of alleles found in a population, *U* is the number of alleles unique to that population, unique alleles occurring at a frequency of ≥ 5% are indicated in parentheses, and % = (*U*/*T*) x 100.

	Belen			San Bernardino		
Locus	*T*	*U*	%	*T*	*U*	%
Scμ 01	7	4 (1)	57.1	10	7 (3)	70.0
Scμ 05	11	3 (2)	27.3	17	9 (5)	52.9
Scμ 07	7	2 (0)	28.6	10	5 (3)	50.0
Scμ 11	9	3 (0)	33.3	16	9 (2)	56.3
Scμ 26	5	0 (0)	0	11	6 (1)	54.6
Scμ 140	13	2 (1)	15.4	15	4 (1)	26.6
Total	52	14 (4)	26.9	79	40 (15)	50.6

proportion of unique alleles in the San Bernardino population may be partly accounted for by the disparity in sample size, such that alleles occurring at low frequency (<5 %) in either population were detected only in the larger San Bernardino sample. Nevertheless, despite a lower sample size, high proportions of unique alleles were detected in the Belen sample at five of six loci, and trends in the proportion of unique alleles among these five loci are roughly parallel between the two populations (Table 12). Interestingly, the Belen population contained no unique alleles at Scμ 26. At this locus, the distribution of alleles in the San Bernardino population was bimodal and the set of low repeat number alleles were absent from the Belen population.

To quantify differentiation between the two populations I calculated standard fixation and distance measures based on the infinite alleles model (F_{ST} and Nei's D). These measures utilize both allelic frequency and identity information to provide an index of differentiation. F_{ST} ranged from 0.014 (Scu 26) to 0.219 (Scu 07) across loci with an overall F_{ST} of 0.127. The overall F_{ST} value was significant ($P = 0.001$, FSTAT permutation procedure). Nei's D was 0.438. I did not calculate fixation and distance measures based on the stepwise mutation model since these loci do not conform to this model. Four loci had bimodal distributions of allele sizes with large gaps between modal frequencies, and although all loci consisted of dinucleotide repeat units, two had alleles that do not seem consistent with increases/decreases in repeat units alone (i.e. single nucleotide differences in length).

Nonrandom associations of alleles

Observed heterozygosity was lower than that expected under Hardy-Weinberg conditions at four of six loci in each population (Table 11). After Bonferroni correction, I found a significant overall heterozygote deficiency in the San Bernardino ($P = 0.005$) population, but not the Belen ($P = 0.042$) population. In locus-population tests I found significant heterozygote deficiency only at Scμ 05 in the San Bernardino population ($P = 0$) after sequential Bonferroni correction.

Population Structure

In the San Bernardino population, encounter rates along the sampling transect were nonrandom and associated with landscape features (Fig. 8). Massasauga were encountered most frequently where the highway crosses rocky bajadas (slopes) associated with large cinder cones. I tested for population structure by dividing the population into three putative demes based on which cinder cone they were associated with: Cinder Pit (0–6.5 km), Red Hill (6.6–11.0 km), or Price Road (11.1–17.0 km). In pair-wise comparisons of allele frequencies between demes I found significant differences only between Cinder Pit and Red Hill demes ($P = 0.005$) following sequential Bonferroni correction. Allele frequencies across loci did not significantly differ between Red Hill and Price Road ($P = 0.030$) or Cinder Pit and Price Road ($P = 0.413$) demes. However, an overall $F_{ST} = 0.006$ was significant ($P = 0.017$, F_{STAT} permutation procedure). Pairwise F_{ST} values among demes Cinder Pit and Red Hill $F_{ST} = 0.010$, Cinder Pit and Price Road $F_{ST} = 0.007$, and Red Hill and Price Road $F_{ST} = -0.008$. Only the Cinder Pit vs. Red Hill F_{ST} value was significant ($P = 0.007$; F_{STAT} permutation procedure).

Using a Mantel test, I found a non-significant negative association (r = -0.0175, t = -0.331, P = 0.3705) between matrices of proportion of total alleles shared between individuals and geographic distance between individuals.

I also calculated the mean ratio of the number of alleles to the range in allele size (M) for the San Bernardino population. This ratio decreases as a result of bottlenecks in populations, because while rare alleles are lost due to genetic drift they are lost randomly throughout the size distribution (Garza and Williamson, 2001). Thus quantitative allelic diversity decreases rapidly, while reductions in spatial diversity take place much more gradually. Simulation models indicate that severe bottlenecks result in reductions in M that persist for over 100 generations (Garza and Williamson, 2001). For the San Bernardino population I calculated M = 0.55, well below the values (0.60 –0.69) reported for eight populations of diploid organisms known to have suffered sometimes severe reductions in population size (Garza and Williamson, 2001). Indeed, other populations, with stable population size histories, all assayed M values above 0.80 (Garza and Williamson, 2001).

Discussion

Population Differentiation

Taken as whole, these results suggest these geographically discrete and isolated populations are also genetically distinct and that a significant portion of the total variation in this subspecies is harbored within isolated populations. Significant F_{ST} values, distance measures, divergent allele frequencies, and elevated proportions of high frequency private alleles all suggest significant divergence. These results are consistent with the

findings of Gibbs et al. (1997), who found that populations as close as 50 km were genetically distinct. Specifically, in pairwise comparisons of five eastern populations of *S. c. catenatus* Gibbs et al. (1997) found F_{ST} values ranged from 0.085–0.261, Nei's D ranged from 0.173–1.133, populations significantly differed in allele frequencies, and proportion of unique alleles ranged from 14.8–32.7% in each population. These data and those of Gibbs et al. (1997) suggest that populations separated by even small distances have been isolated for some time and that gene flow between the populations is very restricted, or in most cases, no longer occurs. Interpreting these data in the context of southwestern geography, the limited vagility of rattlesnakes, habitat requirements of the species and the known distribution of *S. c. edwardsii* strongly suggests absence of extant gene flow between the San Bernardino and Belen populations.

Nonrandom associations of Alleles and Population Structure

In contrast to results from eastern populations (Gibbs et al., 1997) the San Bernardino and Belen populations had relatively low F_{IS} values (Table 13). Combined across loci F_{IS} ranged from 0.118–0.353 in eastern populations, but measured only 0.063 (Belen) and 0.046 (San Bernardino) in southwestern populations. Likewise, I found significant heterozygote deficiencies only in the San Bernardino population, which appears to be primarily due to significant deficiencies at a single locus (Scμ 05). Five eastern populations of *S. c. catenatus* all had significant heterozygote deficiencies and in 33 locus-population comparisons, heterozygote deficiencies were detected 25 times. Heterozygote deficiencies can result from inbreeding, unrecognized population structure and/or null alleles. Gibbs et al. (1997) addressed the possibility of null alleles in their

Table 13. F_{IS} values in two populations of *Sistrurus catenatus edwardsii* by locus as calculated using FSTAT.

Locus	Population	
	Belen	San Bernardino
Scμ 01	0.348	0.018
Scμ 05	0.085	0.137
Scμ 07	-0.139	0.008
Scμ 11	-0.100	-0.011
Scμ 26	0.131	0.000
Scμ 140	0.126	0.113
Combined	0.063	0.046

eastern dataset and concluded they were not the source of heterozygote deficiencies. While this does not preclude the presence of null alleles in other populations, it does suggest low potential for null alleles. In the case of the San Bernardino population I found significantly different allele frequencies and F_{ST} values between the putative Cinder Pit and Red Hill demes, suggesting intra-population structure. I tested the idea that this may be an artifact of isolation by distance along the linear sampling transect using a Mantel test to compare allele-sharing among and geographic distance between individuals. I found a non-significant negative relationship between proportion of alleles shared and geographic distance, lending further credence to the presence of demes or sub-population structure that may be contributing to decreased levels of heterozygosity in the population overall (the Wahlund effect). Data were also examined for the possibility of past or extant bottlenecks in population size using *M* and found evidence of a significant reduction in size of the San Bernardino population. However, the value *M* is the ratio of the number of alleles present in a population to the total "potential" allelic states (measured as the number of repeats possible between the smallest and largest alleles). Four of the loci have bimodal size distributions, which may render the underlying assumptions (*vis a vis* mode of mutation) of this statistic unrealistic.

CONSERVATION IMPLICATIONS

These data suggest that southwestern populations of *S. c. edwardsii* should be monitored and managed as independent entities. It is extremely unlikely that recolonization or migration will ameliorate severe population reductions and extinctions should they occur. Indeed, natural repopulation would probably require an expansion of range and concomitant changes in climate and biotic communities. Translocation of

animals between populations should be considered cautiously (Gibbs et al., 1997), since individual populations are clearly genetically unique. Given that rates of evolution of quantitative genetic variation (with potentially meaningful adaptive relevance) are roughly similar to the rates of evolution at neutral microsatellite loci, data presented here suggests (but does not demonstrate) the potential for local adaptation to different environments.

Intra-population analyses suggest significant structure within the San Bernardino population. These data suggest that the "genetic neighborhood" of *S. c. edwardsii* in the San Bernardino population is associated with habitat features. Management of this population should seek to preserve habitat heterogeneity that supports this diversity. Specifically, disturbance of tobosa grassland communities, of surface features (e.g. cinder cone bajadas), and/or of fauna associated with these communities (e.g. *Dipodomys spectabilis* burrows associated with cinder cone bajadas) may degrade metapopulation structure and function. These data suggest a recent bottleneck in population size. This is consistent with historic vs. extant capture localities which similarly suggest a reduction in population distribution in the San Bernardino valley (ATH, unpubl.). Given the apparently severe effect this bottleneck may have had on allelic diversity in this population, cautious protection of remaining *S. c. edwardsii* habitat to guard against further reductions in genetic variability is necessary.

Isolation and Characterization of Microsatellite Loci from a Threatened Rattlesnake (New Mexico Ridgenose Rattlesnake, *Crotalus willardi obscurus*).

Microsatellite DNA loci have become the genetic marker of choice for studies of paternity, kinship, inbreeding, and fine scale population structure. Alleles are composed of tandem repeats of short sequences (typically 2–4 bp) of nucleotides. Microsatellites are often hypervariable in length (number of repeats) with each length polymorphism comprising a distinct allele for the locus (Tautz, 1989; Quellar et al., 1993). Microsatellites are selectively neutral, co-dominant, inherited in a simple Mendelian fashion, and assignable to specific loci (Quellar et al., 1993; Ashley and Dow, 1994). Furthermore, microsatellite loci often have utility in congeneric species and sometimes even more distantly related species. (e.g. Fitzsimmons et al., 1995; Gibbs et al., 1998; Bushar et al., 2001). Microsatellites have been characterized from a number of other snakes, including *Crotalus horridus* (Villareal et al., 1996), *Sistrurus catenatus* (Gibbs et al., 1998), *Hoplocephalus bungaroides* (Burns and Houlden, 1999), *Nerodia sipedon* (Prosser et al., 1999), *Thamnophis sirtalis* (McCracken et al., 1999), and *Natrix tessellata* (Gautscchi et al., 2000).

Here I report the isolation of 6 microsatellite loci from the New Mexico Ridgenose Rattlesnake *(Crotalus willardi obscurus)*, and describe locus-specific characteristics within a single population. I also assess expected versus observed levels of heterozygosity, the utility of these loci for parentage analyses, and potential for cross-amplification in congeners. *Crotalus w. obscurus* is listed as "threatened" under the U.S.

Endangered Species Act (U. S. Fish and Wildlife Service 1978). These markers will be used for analyses of variation within and among populations of this species, information essential to threat assessment and conservation planning.

METHODS AND MATERIALS

From 1993–1999 ca. 190 tissue samples were collected from *C. w. obscurus* in the Animas Mountains, Hidalgo County, New Mexico. Of these, samples representing 54 individuals were selected for genotyping in this preliminary survey of variability. In addition, I obtained tissue from a litter of eight offspring and their mother in the Peloncillo Mountains, Hidalgo County, New Mexico (Holycross, 2000). All snakes were marked for individual identification using passive integrated transponders (Jemison et al., 1995) to prevent unintentional resampling. Samples of ca. 0.1 ml whole blood were drawn from the caudal vein and immediately stored in either 1 ml 99% ethanol or 1 ml lysis buffer (Seutin et al., 1991). In the case of neonates, sloughed skins were used as a DNA source. Samples were stored at ambient temperatures (ca. 15–37 °C) for up to two months in the field and at <4 °C in the lab. I extracted DNA from ca. 200 µL of whole blood or 1–2 cm^2 of shed skin using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989). DNA was resuspended in 1X TE (pH 7.5) and concentrations were estimated via agarose gel.

Genomic DNA was partially restricted with a cocktail of seven blunt-end cutting enzymes (*Rsa* I, *Hae* III, *Bsr* B1, *Pvu* II, *Stu* I, *Sca* I, *Eco* RV). Fragments of 300 to 750 bp were adapted and subjected to magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey), using biotinylated capture molecules. Libraries were prepared in parallel using

Biotin-CA(15), Biotin-GA(15), Biotin-ATG(12) and Biotin-TAGA(8) as capture molecules in a protocol provided by the manufacturer. Captured molecules were amplified and restricted with *Hind*III to remove the adapters. Resulting fragments were ligated into the *Hind*III site of pUC19. Recombinant molecules were electroporated into *E. coli* DH5alpha. And 192 recombinant clones were randomly selected for sequencing. Sequences were obtained on an ABI 377, using ABI Big Dye terminator cycle sequencing methodology. Primers flanking the repetitive elements were designed using Oligo 4.0 software (National BioSciences Inc., USA) and oligonucleotides were synthesized by MWG Biotech (USA). The forward primer for each pair was labeled with a fluorescent molecule.

PCR amplification for polymorphism assessment was performed in a 20 μL reaction volume containing 10 ng of genomic DNA, 20.0 mM Tris-HCl (pH 9.0), 8.5 mM NaCl, 10 mM KCl, 10.0 mM $(NH_4)^2SO_4$, 2.0 mM $MgSO_4$, 0.1% (w/v) Triton X-100, 0.5% (w/v) Ficoll, 10 picomole each of forward and reverse primer and 0.5 units of Taq DNA polymerase using a PTC-100™ Programmable Thermal Controller (MJ Research Inc.). Amplification was performed under the following conditions: 35 cycles at 95 °C for 30 s, the locus-specific annealing temperature (Table 14) for 30 s, and 72 °C for 30 s. Before the first cycle, a prolonged denaturation step (95 °C for 2 min) was included. Amplified products were diluted with double-distilled water containing GENESCAN-500XL (TAMRA) Size Standard (PE Biosystems) and genotyped on an ABI Prism 377 Genetic Analyser using GeneScanAnalysis® Software version 3.1 and Genotyper® version 2.5 software (PE Biosystems). Observed and expected heterozygosities and likelihood ratio

tests for Hardy-Weinberg equilibrium at each locus were conducted using Popgene version 1.32 (Yeh and Boyle, 1997). Exclusionary power for parentage analyses was conducted using Cervus 2.0 (Marshall et al., 1998).

Results and Discussion

Repetitive sequences were observed in 35 of 192 clones. Primers were designed within flanking sequence and annealing temperatures optimized for 15 clones: 14 of these amplified products. I tested for polymorphism using genomic DNA from 10 individuals. Six of these loci produced scoreable, polymorphic products and were used to survey variation in the Animas Mountain population. Rejected loci were homozygous or difficult to score due to stutter peak configurations. Annealing temperatures, repeat motifs, and primer sequences for each locus are provided in Table 14. Of the 6 microsatellites chosen, four were dinucleotides (*Cw*A14, *Cw*A29, *Cw*B6, *Cw*B23) and two were trinucleotides (*Cw*C24, *Cw*D15). All loci consist of uninterrupted strings of one or two motifs (Table 14). Analysis of a litter of eight *C. w. obscurus* and their mother (from the Peloncillo Mountains) as well as litters of five *Crotalus atrox* and four *Crotalus scutulatus* and their mothers, are not inconsistent with patterns of Mendelian segregation. All offspring share at least one allele with their mother. A maximum of two additional alleles other than those observed in the mother were present among offspring at each locus, which is not inconsistent with single male paternity.

Surveys of 53 to 54 *C. w. obscurus* (sample size varies among loci) from the Animas population revealed 5–24 alleles per locus (Table 14). Although these loci did not exhibit strongly disjunct distributions, most exhibited small gaps between some

TABLE 14. Characteristics of six microsatellite loci developed from *Crotalus willardi obscurus* genomic DNA. F = forward primer and R = reverse primer. * = flourescently labeled primer. T_m = annealing temperature (°C) used to assay variation. A = number of alleles detected. N = number of individuals genotyped. Size refers to the range of allele lengths (bp) detected. H_{exp} = expected heterozygosity (Levene, 1949) and H_{obs} = observed heterozygosity. P = significance level of likelihood ratio (G^2) tests for Hardy-Weinberg equilibrium. F_{IS} = Wright's (1978) fixation index.

Locus	Repeat Motif	Primer sequence (5' → 3')	T_m	Size	A	N	H_{exp}	H_{obs}	P	F_{IS}
*Cw*A14	$(AC)_{24}$	F: GGGGAGGTAGGGAGGTCAG*	62	147–	7	54	0.775	0.68	(0.86)	0.108
		R: AGGGGAAAAGATGCTGTGAG		175				5		
*Cw*A29	$(AC)_{13}$	F: TCCCCTTCCAACCCCCAGA*	60	160–	5	54	0.351	0.31	(0.94)	0.095
		R: CAGAGGAGACGAGACAGATAG		190				5		
*Cw*B6	$(GA)_{19}$	F: CTCTTTTACGCCCACCACTTTA*	56	122–	5	54	0.726	0.79	(0.95)	-
		R: CCCCGCTAACCTTTGCTCAG		130				6		0.107
*Cw*B23	$(TG)_{18}(AG)_{22}$	F: TGGTGTCATCTGGAGTTAAATC*	60	225–	12	53	0.857	0.75	(0.58)	0.111
		R: GCTTTTGTTTATATGGAGAGTCG		271				5		
*Cw*C24	$(CTT)_{49}$	F: ATTGGATAGAAGTAGTTTTGGTA*	62	235–	24	53	0.921	0.90	(1.00)	0.007
		R: CCCCCCTTTTTTTATGGCAGC		313				6		
*Cw*D15	$(CAT)(TAT)(CAT)_{14}$	F: TAATGTTGTAAGCCACCTAGAAT*	58	138–	5	53	0.654	0.71	(0.48)	-
		R: TTCTTCAAAGCACATAACACATC		159				7		0.107

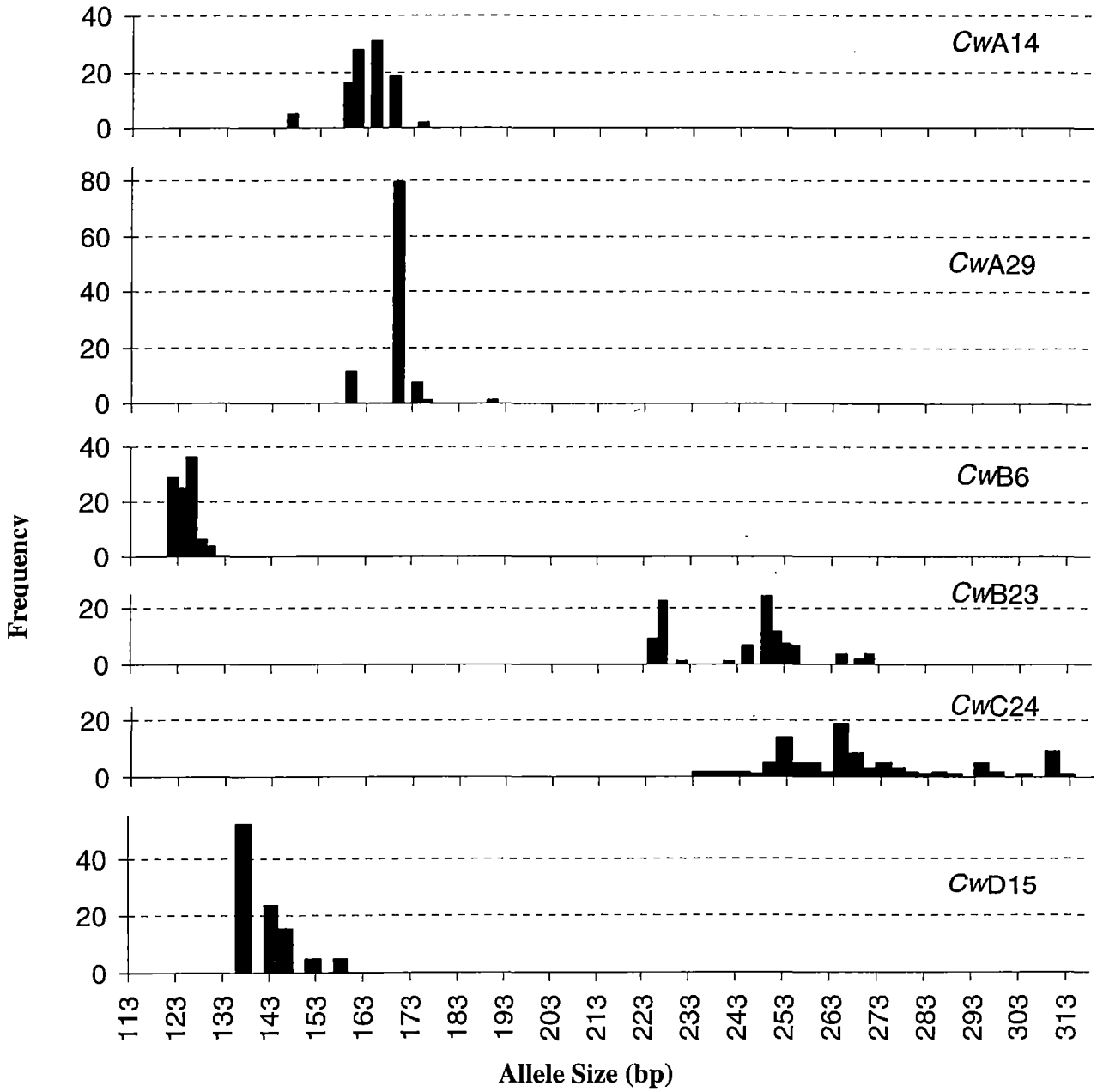

FIG. 9. Allele distribution at 6 microsatellite loci in the Animas population of *Crotalus willardi obscurus*.

adjacent alleles (Fig. 9). Specifically, the largest gaps between alleles were six (*Cw*A29) and five (*Cw*A14) unoccupied potential allelic states. All other loci were characterized by gaps < 4 repeat units between alleles. These distributional patterns are not inconsistent with stepwise (Valdes et al., 1993) or two-phase (Di Rienzo et al., 1994) models of mutation in microsatellites.

Across all loci, mean observed heterozygosity (0.696) approximates mean expected heterozygosity (0.714), and no significant deviations from Hardy–Weinberg expectations were detected (Table 14). These results suggest that the potential for null alleles is low. F_{IS}, a measure of the extent of nonrandom association of alleles within a population, is likewise low (Table 14). I assessed the utility of these loci for parentage analyses by calculating exclusionary power using this dataset of 54 individuals from the Animas population and the program Cervus 2.0 (Marshall et al., 1998). Across all loci, exclusionary power for the first parent is 0.96, whereas if the genotype of one parent is known, exclusionary power for the second parent is 0.99.

I tested the utility of the primers for cross-amplification of homologous loci in four other rattlesnake species using annealing temperatures reported in Table 14. Of 24 locus/species combinations, only three failed to amplify and 18 of the remaining 21 locus/species combinations produced two or more size products (Table 15). Low levels of variability in *C. atrox* and *C. scutulatus* at some loci may be due to a high degree of relatedness among individuals sampled (Table 15). Additional surveys of unrelated individuals and testing using a variety of PCR conditions are necessary for more representative characterization of cross-amplification and levels of polymorphism in

these locus/species combinations. Although loci amplified with heterospecific primers often exhibit reduced variability (Moore et al., 1991; Primmer et al., 1996), these data suggest this set of variable microsatellite loci may prove useful for a variety of population-level and relatedness analyses in *C. willardi* and other rattlesnakes.

TABLE 15. Results of cross-amplification experiments. Sizes of amplified products (in bp) are indicated for each locus. *N* = number of individuals genotyped. *Crotalus atrox* samples consisted of a mother and five offspring as well as one unrelated individual. *Crotalus scutulatus* consisted of a mother and four offspring. *Crotalus lutosus* and *Crotalus tigris* samples consist of unrelated individuals from a single population. Annealing temperatures are the same as those reported in Table 14.

		Locus					
Species	*n*	*Cw*A14	*Cw*A29	*Cw*B6	*Cw*B23	*Cw*C24	*Cw*D15
C. atrox	7	159, 169, 171, 175	170, 182, 184	98, 104	217, 227, 245, 251	–	125
C. lutosus	5	155, 159	166, 172	–	215, 217, 221, 227, 235	–	132, 135, 144, 147, 153
C. scutulatus	5	157, 161	162,164, 166	106	221, 227, 231, 233, 235, 241, 245	259, 262, 298, 307	129, 132, 156
C. tigris	6	157, 165, 167, 169	160, 162	127, 135	233, 241, 243	205, 253, 259, 262, 265, 277, 286, 289, 298	126

Genetic variation and population structure in a threatened rattlesnake (New Mexico Ridgenose Rattlesnake, *Crotalus willardi obscurus*)

Conservation of endangered species requires identification of independently evolving entities or "evolutionarily significant units" (ESUs; Waples 1995) in order to delineate populations and preserve evolutionary history and trajectories. In the case of small isolated populations, additional questions are of interest, such as structure within populations, past demographic history (e.g. bottlenecks) and migration rates. Molecular genetic markers, particularly microsatellite DNA loci, are especially well suited to analysis of fine-scale population structure and differentiation. Microsatellite DNA is often hypervariable in length (number of repeats) with each length polymorphism comprising a distinct allele for the locus (Tautz, 1989; Quellar et al., 1993).

Microsatellites are neutral, co-dominant, inherited in a simple Mendelian fashion, and assignable to specific loci (Quellar et al., 1993; Ashley and Dow, 1994). This suite of characteristics renders them especially useful for studies of relatedness, inbreeding, and recovering recent evolutionary history and extant genetic patterns among closely related populations. They are thus suitable for defining ESUs. Inherent to the concept of ESUs is the ideal of perpetuating adaptive variation among populations. Although microsatellites are neutral, they evolve at rates that approximate rates of evolution of quantitative genetic variation (with potentially meaningful adaptive relevance) and thus can provide insight to the potential for local adaptation (Hedrick et al., 2001).

Crotalus w. obscurus (New Mexico Ridgenose Rattlesnake), is restricted to Madrean montane woodland communities in the Sierra San Luis (Mexico) and in the

neighboring Animas and Peloncillo Mountains (United States). Low elevation passes separate the Sierra San Luis from the Animas and Peloncillo Mountains (Fig. 10) but may have provided corridors for past genetic exchange between the Sierra San Luis and the two northern mountain ranges. The broad Animas Valley separates the Peloncillo and Animas Mountains and is almost certainly a complete barrier to extant migration. Different histories of isolation and rates of reduction in population size may have contributed to different genetic circumstances for each population. Variation was found at three allozymes loci (of 33 screened) in the San Luis population ($N = 6$). However, these loci were monomorphic in small samples from the Animas ($N = 1$) and Peloncillo ($N = 3$; mother and two offspring) populations (Barker, 1992). Several have suggested elaborate phylogeographic and biogeographic scenarios to explain the current distribution of *C. willardi* authors (Fowlie, 1965; Klauber, 1972; McCranie and Wilson, 1987; Barker, 1992). All are variations on a theme of northward expansion of range followed by subsequent vicariance. A fossil from the San Pedro river valley tentatively identified as *Crotalus willardi* (Mead, 1975) lends credence to the hypothesis that *C. w. obscurus* occupied wooded Pleistocene valleys (Barker, 1992) prior to its current insular distribution. *Crotalus w. obscurus* was listed is 'threatened' under the Endangered Species Act (USFWS, 1978). A Species Recovery Plan (Baltosser and Hubbard, 1985) recommended *in situ* study and establishment of a captive breeding program based on extremely limited information.

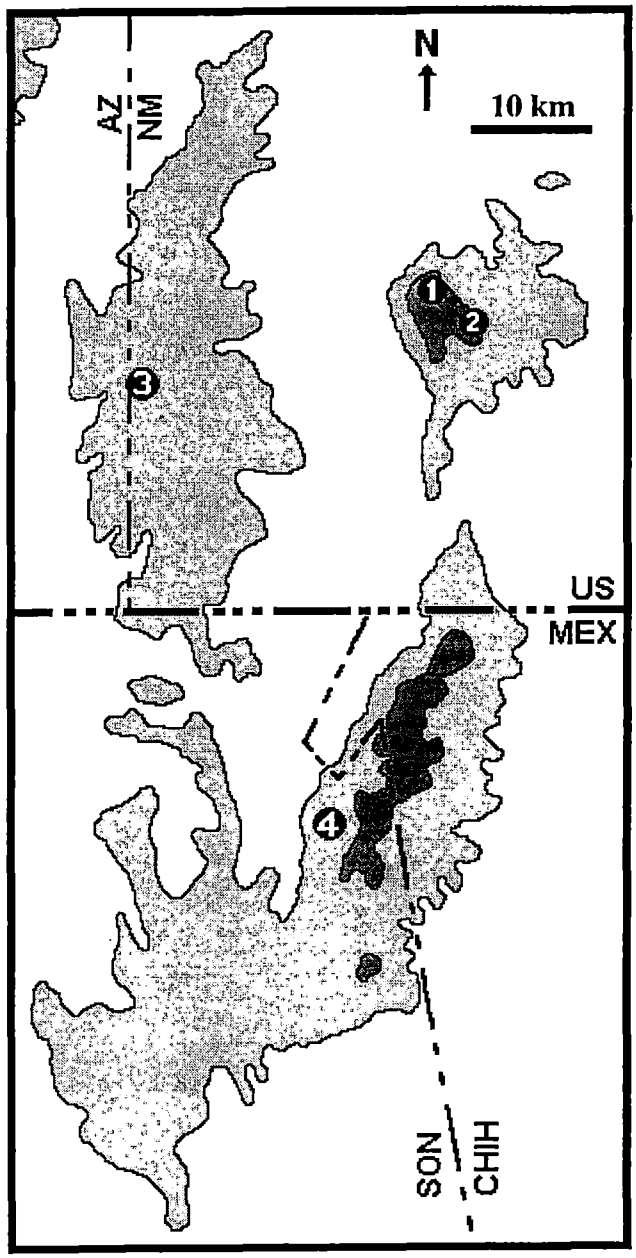

FIG. 10. Location of *Crotalus willardi obscurus* sampling localities in West Fork Canyon, Animas Mountains (1) and Indian Creek Canyon, Animas Mountains (2), Peloncillo Mountains (3), and Sierra San Luis (4). Light and dark gray isopleths delineate Madrean Evergreen Woodland and Petran Montane Conifer Forest, respectively (modified from Brown and Lowe, 1994).

Delineation of patterns of genetic variation within and among populations will allow management agencies to make informed decisions regarding jeopardy rulings, allocation of conservation resources, captive breeding programs, formulation of recovery plans, and translocation of animals. Description of geographic patterns of genetic variation may also help identify source populations of expatriated animals, thus aiding in law enforcement activities and/or repatriation efforts. Currently, Animas Mountain is the only "critical habitat" listed for *C. w. obscurus*. Critical habitat has not been designated for the Peloncillo population, the smallest of the three populations, and the only population occurring on public land. An "exception to jeopardy" ruling by the USFWS is required in order to have separate critical habitat designated for each population. Evidence of independent evolution among populations would facilitate such a ruling. This work is part of a long-term study of the conservation biology of the three known populations of this threatened species (Holycross and Goldberg, 2001; Smith et al., 2001; Holycross et al., In press).

Materials and Methods

Populations Sampled

I sampled tissue from *C. w. obscurus* in the Animas Mountains ($B = 54$), Peloncillo Mountains ($N = 18$), and Sierra San Luis ($N = 29$) from 1993–1999 (Fig. 10). In the Animas Mountains, I sampled from two locations, Indian Creek Canyon (UTM 12: 711286E, 3495126N) and West Fork Canyon (UTM 12: 710728E, 3496594N). Tissues in the Peloncillo Mountains were collected from Clanton Draw (UTM 12: 687984E,

3487552N), Miller Canyon (UTM 12: 686719E, 3483737N), and South Skeleton Canyon (UTM 12, 684083E, 3488934N). In the Sierra San Luis tissues were collected from Junta los Cajones (UTM 12: 699577E, 3450519N), a tributary to Cajon Bonita. Herein, "samples" refers to each of the four sampling localities, while "populations" refers to all samples from each mountain range (i.e. Animas samples combined). To prevent unintentional resampling I marked all snakes for individual identification using passive integrated transponders (Jemison et al., 1995). I sampled ca. 0.1 ml whole blood from the caudal vein and immediately stored this in either 1 ml 99% ethanol or 1 ml lysis buffer (Seutin et al., 1991). In a few cases, skins sloughed during holding were retained as a source of DNA. Due to logistic constraints, samples were stored at ambient temperatures (ca. 15–37 °C) for up to two months in the field. In the laboratory, samples were stored at <4°C, for up to several years.

Laboratory Methods

I extracted DNA from ca. 200 µL of whole blood or 1–2 cm^2 of shed skin using a standard phenol-chloroform extraction protocol (Sambrook et al., 1989). DNA was resuspended in 1X TE (pH 7.5) and concentrations were estimated via agarose gel. Individuals were then genotyped at each of nine microsatellite loci; six (*Cw*A14, *Cw*A29, *Cw*B6, *Cw*B23, *Cw*C24, *Cw*D15) developed from *Crotalus willardi obscurus* genomic DNA (Chapter 8) and three loci (Scµ01, Scµ07, Scµ11) from *Sistrurus catenatus* (Gibbs et al., 1998). Two loci are trinucleotide repeats (*Cw*C24, *Cw*D15) and the remaining seven are dinucleotide repeas. PCR amplification for polymorphism assessment was performed in a 20 µL reaction volume containing 10 ng of genomic DNA, 20.0 mM Tris-

HCl (pH 9.0), 8.5 mM NaCl, 10 mM KCl, 10.0 mM $(NH_4)^2SO_4$, 2.0 mM $MgSO_4$, 0.1% (w/v) Triton X-100, 0.5% (w/v) Ficoll, 10 picomole each of forward and reverse primer and 0.5 units of Taq DNA polymerase thermotreatment on a PTC-100™ Programmable Thermal Controller (MJ Research Inc.): 35 cycles at 95 °C for 30 s, the locus-specific annealing temperature (Holycross et al., in prep.) for 30 s, and 72 °C for 30 s. A prolonged denaturation step (95 °C for 2 min) was included before the first cycle. Amplified products were diluted with double-distilled water containing GENESCAN-500XL (TAMRA) Size Standard (PE Biosystems) and analyzed on an ABI Prism 377 Genetic Analyser using GeneScanAnalysis® Software version 3.1 and Genotyper® version 2.5 software (PE Biosystems).

Analysis of Variation

Initial manipulations of data, data checking, and creation of input files was conducted using the MS Toolkit macro for Excel (Park, 2001). I calculated allele frequencies and expected and observed heterozygosity using POPGEN version 1.32 (Yeh and Boyle, 1997). Randomization tests for Hardy-Weinberg equilibrium were conducted using GENEPOP version 3.1 (Raymond and Rousett, 1995). I tested each locus in each of the three populations (27 comparisons) and in each of the samples from the Animas Mountains (18 comparisons). GENEPOP combines these probabilities to test populations across loci or one locus across populations. Critical values for statistical significance were Dunn-Šidák adjusted (Sokal and Rohlf, 1995) for multiple comparisons.

I quantified differentiation between populations using several methods. I tested for heterogeneity in allele frequencies among and between populations (and between

Animas samples) using the exact test in GENEPOP. Proportions of private alleles in each population were calculated by hand. Fixation (F_{ST}; Weir and Cockerham, 1984) and distance indices (D; Nei, 1978) based on the infinite alleles model (IAM) were calculated using GENEPOP and POPGEN, respectively. PHYLIP (Felsenstein, 1993) was used to construct a UPGMA (unweighted pair-group method using arithmetic averages) tree representing the phylogenetic relationships among sampling sites based on genetic distance. FSTAT version 2.9.1 (Goudet. 1995) was used to calculate F-statistics (F_{IS}, F_{ST}, and G'_{ST}) and test F_{ST} estimators for significance. Nm was estimated using the private alleles method (Barton and Slatkin, 1986) in GENEPOP.

I used the program BOTTLENECK version 1.2.02 (Cornuet and Luikart, 1996) to assess statistical evidence for a past bottleneck in each of the three populations. Due to small sample size and few loci, I used the Wilcoxon signed-ranks option in BOTTLENECK to test for differential rate of decline in number of alleles relative to heterozygosity under the two-phase mutation model (TPM) in a population at equilibrium (H_{eq}). I also assayed for evidence of recent bottlenecks using M (Garza and Williamson, 2001), a ratio of the number of alleles detected relative to range in allele sizes. I calculated M for each population using the six loci (*Cw*A14, *Cw*A29, *Cw*B6, *Cw*B23, *Cw*C24, *Cw*D15) that conformed to calculation criteria and were developed from *C. w. obscurus* genomic DNA.

RESULTS

Allele frequencies for all locus-sample combinations are reported in Appendix F. Genetic characteristics and summary statistics for each locus-population combination are

TABLE 16. Genetic characteristics of three populations (and two subsamples) of *Crotalus willardi obscurus* at nine microsatellite loci. ICC and WFC indicate Indian Creek Canyon and West Fork Canyon demes. N = number of individuals genotyped. A = number of alleles detected. 'Size' = the range of allele lengths (bp) detected. H_{obs} = observed heterozygosity and H_{exp} = expected heterozygosity (Levene, 1949). P = significance level of randomization tests for Hardy-Weinberg equilibrium. F_{IS} = Wright's (1978) fixation index.

	Population				
Locus	San Luis	Peloncillo	Animas (pooled)	Animas (WFC)	Animas (ICC)
***Cw*A14**					
N	29	18	54	30	24
A	8	3	7	6	6
Size	147–167	165–169	147–175	147–175	147–169
H_{obs}	0.793	0.444	0.685	0.633	0.750
H_{exp}	0.848	0.513	0.775	0.758	0.778
P	0.138	0.376	0.658	0.394	0.774
F_{IS}	0.065	0.137	0.117	0.167	0.036

continued

TABLE 16, continued

***Cw*A29**					
N	29	18	54	30	24
A	11	5	5	3	5
Size	160–196	172–190	160–190	160–174	160–190
H_{obs}	0.759	0.833	0.315	0.300	0.333
H_{exp}	0.752	0.675	0.351	0.297	0.420
P	0.548	0.881	0.473	0.593	0.339
F_{IS}	–0.009	-0.244	0.104	-0.012	0.210
***Cw*B6**					
N	29	18	54	30	24
A	7	4	5	5	4
Size	98–128	98–134	122–130	122–130	122–128
H_{obs}	0.759	0.444	0.796	0.767	0.833
H_{exp}	0.756	0.592	0.726	0.740	0.705
P	0.728	0.445	0.964	0.997	0.758
F_{IS}	-0.003	0.255	-0.098	-0.037	-0.187

continued

TABLE 16, continued

***Cw*B23**					
N	29	18	53	29	24
A	13	7	12	10	11
Size	245–275	233–271	225–271	225–271	225–271
H_{obs}	0.724	0.667	0.755	0.793	0.708
H_{exp}	0.868	0.783	0.857	0.849	0.865
P	0.025	0.032	0.070	0.459	0.006
F_{IS}	0.168	0.152	0.121	0.067	0.185
***Cw*C24**					
N	29	18	53	30	23
A	18	15	24	18	22
Size	229–319	229–304	235–313	235–313	235–310
H_{obs}	0.966	0.778	0.906	0.900	0.913
H_{exp}	0.905	0.932	0.921	0.902	0.942
P	0.718	0.207	.423	0.286	0.300
F_{IS}	-0.068	0.169	0.017	0.003	0.031

continued

TABLE 16, continued

***Cw*D15**					
N	29	18	53	29	24
A	7	5	5	5	5
Size	132–156	138–156	138–159	138–159	138–159
H_{obs}	1.000	0.889	0.717	0.793	0.625
H_{exp}	0.820	0.735	0.654	0.684	0.614
P	0.275	0.981	0.814	0.492	0.842
F_{IS}	-0.225	-0.217	-0.097	-0.162	-0.018
Scμ01					
N	28	18	53	29	24
A	9	4	11	9	10
Size	158–208	178–204	166–204	166–204	166–204
H_{obs}	0.786	0.667	0.906	0.966	0.833
H_{exp}	0.781	0.560	0.849	0.828	0.864
P	0.054	0.616	0.270	0.583	0.312
F_{IS}	-0.006	-0.196	-0.068	-0.169	0.037

continued

TABLE 16, continued

Scμ07					
N	28	13	52	29	23
A	1	1	2	2	2
Size	146	146	146–148	146–148	146–148
H_{obs}	0	0	0.539	0.621	0.435
H_{exp}	0	0	0.505	0.508	0.510
P	—	—	0.781	0.278	0.676
F_{IS}	—	—	-0.067	-0.226	0.151
Scμ11					
N	27	18	53	29	24
A	12	6	10	9	10
Size	170–216	194–210	172–214	172–212	172–214
H_{obs}	0.852	0.667	0.736	0.759	0.708
H_{exp}	0.851	0.735	0.861	0.836	0.892
P	0.210	0.873	0.022	0.024	0.135
F_{IS}	-0.002	0.095	0.146	0.093	0.209

continued

TABLE 16, continued

All loci					
N (mean)	28.6	17.4	53.2	29.4	23.8
A (mean)	9.6	5.6	9	7.4	8.3
H_{obs}	0.738	0.599	0.706	0.726	0.682
H_{exp}	0.731	0.614	0.722	0.711	0.732
P	0.063	0.516	0.300	0.360	0.176
F_{IS}	-0.009	0.025	0.022	-0.021	0.070

provided in Table 16. Twenty-five (93%) of 27 locus/population combinations were polymorphic. The only monomorphic exceptions were the San Luis and Peloncillo populations at *Scu*07. *Scu*07 had the lowest expected and observed heterozygosity, the lowest average number of alleles across populations (1.3), and just two alleles detected overall (Table 16). *Cw*C24 had the highest expected and observed heterozygosity, the highest average number of alleles across populations (19.0), and the most alleles (across populations) with 30 alleles occupying 31 possible trinucleotide positions between 229–319 bp (Table 16).

Populations

Although all populations contained substantial genetic variation, the Peloncillo population was less variable than the Animas or San Luis populations. The Peloncillo population averaged 5.6 alleles/locus, compared with 9.0 and 9.6 alleles/locus in the Animas and San Luis populations. Average expected heterozygosity ranged from a low of 0.61 in the Peloncillo population to 0.72 and 0.73 in the Animas and San Luis populations, respectively. After correction of critical values for multiple comparisons, no significant departures from Hardy–Weinberg expectations were detected in locus specific tests within populations (Table 16), in populations (across loci), or at individual loci (across populations). I also calculated the "inbreeding coefficient" (F_{IS}), which is a measure of the heterozygote deficit within populations, for each locus in each population and sample (Table 16). Overall F_{IS} did not significantly differ from zero (bootstrapping across loci 99% confidence intervals: –0.075 to 0.089). I tested for bottlenecks in each population using the Wilcoxson signed-rank test to compare observed heterozygosity to

H_{eq} under the TPM (Cornuet and Luikart, 1996) and detected significant differences only in the Peloncillo population ($P < 0.02$). Evidence of bottlenecks was also assessed by calculating M (Garza and Williamson, 2001) for each population: $M = 0.653 \pm 0.059$ in the San Luis population, 0.632 ± 0.108 in the Animas population, and 0.559 ± 0.114 in the Peloncillo population.

The Animas Mountains population was sampled from two geographically discrete canyons in order to facilitate analysis of genetic patterns within mountain ranges. Allele frequencies in the two Animas samples did not significantly differ in any locus-specific comparison after correction for multiple comparisons (Table 17). Nor did they significantly differ when combined across loci ($P = 0.051$). While there were unique alleles in each of these samples (when contrasted exclusively with each other), the frequency of unique alleles was generally low and these typically appeared at loci where the number of alleles detected approached the sample size (Appendix F). Observed heterozygosity was slightly lower than, but not significantly different from expected heterozygosity in the population overall (Table 16), and F_{IS} did not significantly differ from zero. Genetic distance between the WFC and ICC samples in the Animas Mountains was low (0.035) as was F_{ST} (0.004). Measures of migration based on pairwise F_{ST} (that require a number of biologically unreasonable assumptions; Whitlock and McCauley, 1999) estimate Nm at 62.3. Using the private allele method to estimate migration rates between the two samples I found $Nm = 3.04$, after correction for size (Barton and Slatkin, 1986).

TABLE 17. Significance levels of tests of Ho: allele frequencies do not differ among/between populations. A = Animas, P = Peloncillo, and S = San Luis populations. ICC and WFC indicate the Indian Creek Canyon and West Fork Canyon samples in the Animas Mountains.

	A v. P v. S	A v. P	P v. S	S v. A	ICC v. WFC
Locus	*P*	*P*	*P*	*P*	*P*
*Cw*A14	0.000	0.000	0.000	0.000	0.089
*Cw*A29	0.000	0.000	0.000	0.000	0.422
*Cw*B6	0.000	0.000	0.025	0.000	0.192
*Cw*B23	0.000	0.000	0.000	0.000	0.293
*Cw*C24	0.000	0.030	0.000	0.000	0.228
*Cw*D15	0.000	0.000	0.000	0.000	0.256
Scμ01	0.000	0.000	0.000	0.000	0.021
Scμ07	0.000	0.000	—	0.000	0.843
Scμ11	0.000	0.000	0.000	0.000	0.246
Combined	**0.000**				**0.051**

Population Differentiation

Allele frequencies among the three populations significantly differed at all loci ($P = 0$ for all comparisons) and when combined across loci ($P = 0$; Table 17). In pair-wise comparisons of allele frequencies at each locus, most tests (25 of 27) were highly significant ($P = 0$). Two tests were non-significant after critical values were adjusted for multiple comparisons: the Peloncillo and San Luis populations at *Cw*B6 and the Animas and Peloncillo populations at *Cw*C24 (Table 17). Unique and private alleles were detected in all three populations (Table 18). Private alleles are unique alleles with frequencies $\geq 5\%$. In the Animas and San Luis populations, 30% and 37 % of alleles were unique and 12% and 20% of alleles were private. In contrast, 14% of alleles in the Peloncillo population were unique and 8% of alleles were private. The relatively low proportion of unique alleles in the Peloncillo population may be partly accounted for by low sample size, such that alleles occurring at low frequency (<5 %) were not detected. However, in both the San Luis and Animas populations, 5% of all alleles were private alleles that occurred at frequencies of ca. 20% or higher, whereas none of the private alleles in the Peloncillo approached these proportions. Furthermore, in a comparison of 18 individuals drawn at random from each population, five private alleles were detected in the Peloncillo population, whereas 10 and 18 private alleles were detected in the Animas and San Luis populations.

Genetic distance between mountain populations (Table 19) ranged from 0.446 (Peloncillo - San Luis) to 0.780 (Peloncillo- Animas). Pair-wise distances between all four samples (Table 19) were used to construct a UPGMA phylogenetic tree to

TABLE 18. Distribution of alleles unique to each population of *Crotalus willardi obscurus*. T = total number of alleles from a population, U is the number of alleles unique to the population, and the number of these that occur at a frequency ≥ 5% is indicated in parentheses, and % = (U/T) x 100.

	San Luis			Peloncillo			Animas		
Locus	*T*	*U*	%	*T*	*U*	%	*T*	*U*	%
*Cw*A14	8	4 (4)	50	3	1 (1)	33	7	2 (1)	29
*Cw*A29	11	6 (3)	55	5	1 (1)	20	5	1 (0)	20
*Cw*B6	7	2 (1)	29	4	1 (0)	25	5	1 (0)	20
*Cw*B23	13	5 (1)	39	7	3 (2)	43	12	4 (2)	33
*Cw*C24	18	5 (3)	28	15	1 (0)	7	24	6 (0)	25
*Cw*D15	7	2 (2)	29	5	0	0	5	1 (1)	20
Scμ01	9	3 (1)	33	4	0	0	11	5 (4)	46
Scμ07	1	—	—	1	—	—	2	1 (1)	50
Scμ11	12	5 (2)	42	6	0	0	10	3 (2)	30
Total	**86**	**32 (17)**	**37**	**50**	**7 (4)**	**14**	**81**	**24 (10)**	**30**

TABLE 19. Measures of population differentiation among samples and populations of *Crotalus willardi obscurus*. F_{ST} (Weir and Cockerham, 1984) is above the diagonal and genetic distance (Nei, 1978) is below the diagonal. ICC = Indian Creek Canyon and WFC = West Fork Canyon in the Animas Mountains.

	Animas (WFC)	Animas (ICC)	Peloncillo	San Luis
Animas (WFC)	—	0.004	0.211	0.139
Animas (ICC)	0.035	—	0.203	0.129
Peloncillo	0.788	0.788	—	0.144
San Luis	0.558	0.544	0.446	—

	Animas	Peloncillo	San Luis
Animas	—	0.203	0.133
Peloncillo	0.780	—	0.144
San Luis	0.543	0.446	—

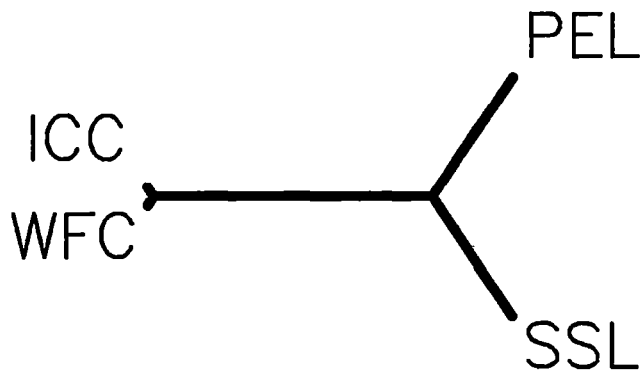

FIG. 11. UPGMA phylogenetic tree of relationships among *Crotalus willardi obscurus* populations based on genetic distance (Nei, 1978). ICC and WFC represent the Indian Creek Canyon and West Fork Canyon samples in the Animas Mountains, while PEL and SSL represent the Peloncillo Mountains and Sierra San Luis samples.

summarize relationships among samples (Fig. 11). F_{ST} (Weir and Cockerham, 1984) was 0.1594 and G'_{ST} (Nei, 1987) was 0.164. Bootstrapping across loci, the 99% confidence interval for overall F_{ST} ranged from 0.086–0.255. Pairwise F_{ST} estimates for each locus are presented in Table 19. Using the private allele method (Barton and Slatkin, 1986) and after correction for size, $Nm = 0.66$ among the three populations.

DISCUSSION

Populations

On the whole, these microsatellite loci exhibited high levels of variability, rendering them useful for investigations of population structure and differentiation. All populations conformed to Hardy-Weinberg equilibrium, facilitating uncomplicated analysis and interpretation. For example, conformation to Hardy-Weinberg expectations and absence of significant F_{IS} values, suggest that null alleles, a common problem in microsatellite studies, are not a factor in this dataset. In comparisons between the West Fork Canyon and Indian Creek Canyon samples of the Animas Mountains, homogeneity of allele frequencies, minimal genetic distance, and exceptionally low F_{ST} all suggest little or no population genetic structure. Estimates of migration rate appear to differ dramatically, but from a functional perspective, $Nm = 3$ is more than sufficient to effect homogeneity of allele frequencies. Migration rates within the Sierra San Luis are probably comparable to the Animas Mountains, given the continuity and high quality of habitat in the former. Migration rates within the Peloncillo population may not be comparable to values calculated for the Animas population, because population densities

are probably much lower (see below), habitat is of lower quality, and habitat patches are much more fragmented and widely spaced.

Comparison of number of alleles detected, average expected heterozygosity, and private alleles among populations all suggest decreased variability in the Peloncillo population relative to the other two populations. Additionally, the Peloncillo population tested positive in two statistical assays for population bottlenecks. Both methods are based on the TPM, although the method of Cornuet and Luikart (1996) relies on differential rate of decline in two measures (see above) using a mutation model-dependent (TPM in this case) estimation, whereas the method of Garza and Williamson (2001) compares two measures directly and contrasts the ratio with a critical value (M_c) from a user-specified parameterization of the TPM. Both methods require stout sample sizes in order to have much power, and the Peloncillo sample size is marginal for either test. A minimal sample of 15 individuals and 10 loci is recommended for the Wilcoxon test in BOTTLENECK and a sample of 25 or twice the number of alleles at the most polymorphic locus is recommended for M. I calculated M_c using two different sets of mutation parameters. In the first case, I set the proportion of stepwise mutations (p_s) to 0.90 and the average increase in non-stepwise mutations (Δ_g) to 3.5 (parameters recommended by the authors). For a more conservative comparison, I set $p_s = 0.80$ and left $\Delta_g = 3.5$. In all cases $\theta = 10$ and sample size and number of loci were fixed. Under these parameterizations, $M_c = 0.616$, 0.681, and 0.648 (recommended) and 0.542, 0.623, and 0.580 (conservative) for the Peloncillo, Animas, and San Luis populations, respectively. Using the parameterization recommended by the authors, the Peloncillo and Animas populations both tested positive for recent bottlenecks. None of the populations

tested positive under the more conservative parameterization. Garza and Williamson (2001) presented measures of the statistic *M* for 12 natural populations with stable histories and eight populations with documented histories of severe population reductions or founder events. Stable populations ranged from 0.823–0.926 whereas founder, island or reduced populations ranged from 0.599–0.693. The values of *M* for all three *C. w. obscurus* populations fall in the latter range, with the exception of the Peloncillo population, which falls below it. As a whole these tests suggest that reductions in population size have, or are, reducing variability in the Peloncillo population, although given the uncertain mode of microsatellite mutation and the limited sample size, it is not possible to characterize the nature and extent of this decline.

Interestingly, under all but the most conservative parameterizations of TPM, the Animas population also tested positive for bottlenecks using *M* and approached significance ($P = 0.08$) in the Wilcoxon test. The Animas Mountains are higher in elevation than the Peloncillo Mountains, and have higher quality habitat but are the most limited in area (< 30 km^2 of habitat). Thus, this population might be more vulnerable to stochastic environmental catastrophes such as fire or prolonged and severe drought.

Despite decreased variability relative to its neighbors and possible bottlenecks, the Peloncillo population is not genetically impoverished at these neutral markers. Some of these measures (e.g. number of alleles) are sensitive to low sample size (especially with highly variable loci). In addition, overall F_{IS}, a measure of inbreeding (in the absence of null alleles and Wahlund effects), is not unreasonably high and is comparable with overall F_{IS} in the Animas Mountains. Indeed, samples in the Peloncillo Mountains were collected over several canyons (as compared to one canyon in the Sierra San Luis

and two canyons in the Animas Mountains), raising the possibility that a Wahlund effect is contributing to F_{IS} in the Peloncillo and Animas populations. Regardless of genetic diversity, low capture rates (294 person-hours/snake) suggest a very low-density population in the Peloncillos, comprised of few snakes dispersed among fragmented habitat. Capture rates in the Animas Mountains (37 person-hours/snake) and Sierra San Luis (11 person-hours/snake) were much higher than those recorded in the Peloncillo Mountains. Although strong evidence for inbreeding in the Peloncillo is lacking at these neutral markers, I found that two (of 18) individuals (11%) captured in recent years exhibited abnormalities of the rattle that appear to be congenital. I have not observed rattle abnormalities in the Animas Mountains (N = 160) or Sierra San Luis (N = 29).

Even at moderate densities, selection in rattlesnake mating systems appears to act significantly on male mate-searching abilities (Duvall et al., 1992). In low density fragmented populations, where the ability to find mates is compromised, an Allee Effect (Allee, 1958) does not seem improbable. Anecdotal evidence suggests that mate-finding in the Peloncillo population is problematic. First, the population was discovered from a *C. willardi* X *lepidus* hybrid (Campbell et al. 1989), the only documented natural hybrid between these two broadly sympatric and syntopic species. Rare hybridization events between syntopic taxa often result from breakdown of pre-mating isolating mechanisms; a consequence of low population density in one of the two taxa. Paternity analysis of a litter of eight born in the Peloncillo offers a second line of evidence that the pool of potential mates in the Peloncillo population is small. Using genotypes from this litter and all male snakes captured in Clanton Draw, I calculated a 98% probability of paternity for a male snake captured the previous year in the same canyon. Over the course of four

years, only two male snakes had been captured in this drainage, and one of these sired the litter. Intercanyon migration in the Peloncillo Mountains may be essential for population viability in a fragmented low-density system.

Population Differentiation

All measures of population differentiation illustrate that a high proportion of genetic variance in *C. w. obscurus* is partitioned among geographically discrete island populations. All loci significantly differed in allele frequencies among populations and all had high levels of unique and private alleles, suggesting that these populations have been isolated long enough to allow genetic drift to effect significant divergence. While a high proportion of unique alleles can suggest significant divergence between populations, it can also result from missing low frequency alleles due to inadequate sampling of alleles ($2N$) relative to the number of alleles detected overall. In this study, allelic diversity was high, but generally was less than a quarter the number of alleles sampled, with the exception of locus *Cw*C24 (Table 16). Nevertheless, in order to account for this effect I contrast the number of private alleles found in this study with previous studies. Private alleles occurred in proportions equivalent to those reported for Eastern Massasauga (Gibbs et al., 1997). In the aforementioned analysis of microsatellite variation among 6 populations of Bighorn Sheep, Gutiérrez-Espeleta et al. (2000) did not detect any private alleles.

Overall F_{ST} for these populations (0.16) is relatively high when considered in the context of the results of other microsatellite studies at wider spatial scales. For example, Gibbs et al. (1997) obtained an overall F_{ST} of 0.164 in a comparisons among five

populations of Massasauga rattlesnakes from the Great Lakes region, with the furthest populations over 600 km apart. Likewise, Massasauga from the desert southwest, separated by 410 km had an F_{ST} of 0.127 (data reported in Chapter 8). Gutiérrez-Espeleta et al. (2000) found that $F_{ST} = 0.204$. The samples referenced herein are separated by < 50 km in all cases, and the Sierra San Luis is separated from both the Peloncillo and Animas mountains by as little as 5–10 km of unsuitable habitat. Genetic distances calculated here are likewise high, when considered in the context of the literature. For example, Gibbs et al. (1997) calculated a distance of 0.799 between the Cicero, New York and Springfield, Ohio populations of Massasauga, while I calculate a comparable distance of 0.780 between the Animas and Peloncillo populations. A linear distance of 10 to 20 km separates the Animas and Peloncillo populations, whereas the two Massasauga populations are separated by over 600 km.

Relationships among populations as hypothesized in the unrooted UPGMA tree based on genetic distance should be interpreted carefully. Although the Animas samples are clearly most closely related to one another, the relationships among the three montane populations are less certain. Genetic distance increases linearly with time since divergence of two populations. However, genetic distance is sensitive to population size, and can increase rapidly if one or both of the populations experiences a significant decrease in population size (Hedrick, 1999). In the present example, the Peloncillo population exhibits reduced diversity and the statistic *M* suggests a recent bottleneck, creating a context for inflated genetic distance measures in pair-wise comparisons. Nevertheless, pair-wise genetic distances between the populations suggest that the Sierra San Luis and Peloncillo populations are more closely related than the Animas and San

Luis populations and that the Animas and Peloncillo populations are most distantly related. Even if low sample size in the Peloncillo population is inflating distance measures in pair-wise comparisons, it appears that the trend among populations is valid. These patterns fit intuitive expectations given the biogeography of the region. The Peloncillo Mountains and Sierra San Luis are separated only by a series of low (ca. 1670 m) dissected hills currently dominated by a mosaic of grassland and oak woodland that is marginal habitat for *C. w. obscurus*. Given paleoecological evidence from the region (Chapter 2), it seems reasonable that within the last 10,000 years these hills supported more extensive woodlands and possibly a contiguous San Luis-Peloncillo population of *C. w. obscurus*. A low elevation (1677 m) pass dominated by desert scrub and xeric grassland may provide a more extensive barrier between the Sierra San Luis and Animas Mountains. Accordingly, the vicariant event separating the latter populations may have occurred earlier, perhaps near the end of the last pluvial period, if not before. In addition to their association with woodlands, *C. w. obscurus* is saxicolous and seldom found far from rocks (Armstrong and Murphy, 1979; McCranie and Wilson, 1987; Holycross et al., in press). Consequently these rocky intermontane passes may have provided reasonable habitat for the species prior to xerification and changes in the biotic community. In contrast, the broad flat grasslands of the Animas Valley separate the Animas and Peloncillo mountains. This barrier is substantially lower in elevation (1500 m) and more sustained than the aforementioned mountain passes. Even if woodlands covered the Animas Valley during recent glacial episodes, it is uncertain whether or not the basin would have afforded suitable habitat due to the lack of other habitat components such as rocks and slopes.

Most intraspecific phylogenies and phylogeographic scenarios (McCranie and Wilson, 1987; Barker, 1992; K. Zamudio, pers. comm.) place *C. w. obscurus* as sister to *C. w. silus* and/or suggest that *C. w. obscurus* is a recently derived subspecies in the clade. *Crotalus w. obscurus* is separated from *C. w. silus* (in the Sierra el Tigre and beyond) by a low elevation valley cut by the Rio Bavispe. The two subspecies differ dramatically in background coloration and facial patterns, suggesting significant divergence. When ancestral *C. willardi* crossed the Rio Bavispe and occupied the Sierra San Luis, barriers to further dispersal into the Animas and Peloncillo Mountains should have been minimal to nonexistent. In the context of 1) the biogeographic history of the region, 2) the low vagility and habitat specificity of the organism and 3) these population genetic data, vicariance seems a more probable explanation of the genetic diversity observed among populations than colonization of the Animas and Peloncillo populations across barriers. Although allele frequencies differ significantly, the distribution of allele sizes overlaps broadly among populations, and diminished allelic diversity in the Peloncillo population generally occurs throughout the range of allele sizes or is a subset of variation found in the San Luis population. In the context of the generation lengths (ca. 3 years) and mutation rates (ca. 5×10^{-4}/locus/generation) that apply here, if *C. willardi* crossed barriers to occupy the two northern ranges, then colonized populations should exhibit reduced variability due to founder effects and/or show evidence of divergent allele distributions at some loci. Subsequent to invasion of this V-shaped complex of mountain ranges, tips of the "V" may have begun to diverge *in situ*, with subsequent vicariant events creating the context for independent evolution of all three populations. Estimation of migration rate among populations is low, with $Nm = 0.66$, or less than one

migrant/generation. The private alleles method predicts a decrease in log*Nm* as a linear function of the average frequency of private alleles. This method does not demonstrate gene flow is occurring, but rather provides an index of migration rates assuming that gene flow is occurring. Low *Nm*, such as that estimated here, is not inconsistent with the absence of extant gene flow.

Conservation Implications

These data, biogeographic history, and the vagility and habitat specificity of this species all suggest that the populations occupying these three mountain ranges are genetically isolated and currently on independent evolutionary trajectories. Variation at these neutral loci is largely a consequence of genetic drift. Although this study does not demonstrate adaptive variation among populations, it predicts substantial potential for local adaptation, provided that selection pressures vary significantly among populations (Hedrick, 1999; Hedrick et al., 2001). Disparate ecological conditions in the Peloncillo Mountains relative to the Sierra San Luis and Animas Mountains suggest that this is not an unreasonable assumption (see Chapters 2 and 6). For these reasons, I suggest each population be regarded as a distinct evolutionary lineage, or ESU. Data from the Animas Mountains suggests genetic cohesiveness and high levels of gene flow within mountain ranges, although this generality may not apply to the Peloncillo Mountains.

The genetic status of the Peloncillo population is difficult to ascertain. Comparatively low levels of diversity in several genetic parameters and weak statistical evidence for a population bottleneck raise the possibility of recent moderate or chronic mild reductions in population size. Regardless of genetic variability, field studies suggest this population is exceptionally small. While transplantation of snakes from neighboring

populations does not appear to be necessary or advisable from a genetic perspective, such management actions might be necessary from a demographic perspective (see Lande, 1988). These data and the biogeography of this mountain complex suggest that the San Luis population is the most suitable source population in the event demographic supplementation is necessary. Exceptionally low population densities and fragmented habitat suggest that maintaining habitat patches is probably essential to population viability.

LITERATURE CITED

ADLER, K. K. 1960. On a brood of *Sistrurus* from northern Indiana. Herpetologica 16:38.

ALDRIDGE, R. D. 1979a. Female reproductive cycles of the snakes *Arizona elegans* and *Crotalus viridis*. Herpetologica 35:256–261.

ALDRIDGE, R. D. 1979b. Seasonal spermatogenesis in sympatric *Crotalus viridis* and *Arizona elegans* in New Mexico. Journal of Herpetology 13:187–192.

ALLEE, W. C. 1958. The Social Life of Animals. Rev. ed. Beacon Press, Boston.

ANDERSON, R. S., AND T. R. VAN DEVENDER. 1991. Comparison of pollen and macrofossils in packrat (Neotoma) middens: a chronological sequence from the Waterman Mountains of southern Arizona, U.S.A. Review of Paleobotany and Palynology 68: 1–28.

ANDRADE, D. V., AND A. S. ABE. 1999. Relationship of venom ontogeny and diet in *Bothrops*. Herpetologica 55:200–204.

ANDREN, C., AND G. NILSON. 1983. Reproductive tactics in an island population of adders, *Vipera berus* (L.), with a fluctuating food resource. Amphibia-Reptilia 4:63–79.

ANTEVS, E. 1955. Geological-climatic dating in the West. American Antiquity 20: 317–355.

APPLEGARTH, J. S. 1980. The ridge-nosed rattlesnake in New Mexico: A review of existing information and a search for suitable habitat on public lands. Unpubl. Rept., U.S. Bureau of Land Management, Las Cruces, New Mexico. 140 pp.

APPLEGATE, R. D. 1995. *Sistrurus catenatus catenatus*: Food habits. Herpetological Review 26:206.

ARMSTRONG, B. L., AND J. B. MURPHY. 1979. The Natural History of Mexican Rattlesnakes. University of Kansas Museum of Natural History Special Publication No. 5.

ARNOLD, S. 1993. Foraging theory and prey-size predator-size relations in snakes. *In* R. A. Seigel and J. T. Collins (eds.), Snakes: Ecology and Behavior, pp. 87–115. McGraw-Hill, New York.

ASHLEY, M. V., AND B. D. DOW. 1994. The use of micosatellite analysis in population biology: Background, methods and potential applications. *In* B. Schierwater, B. Streit, G. P. Wagner and R. DeSalle (eds.), Molecular Ecology and Evolution: Approaches and Applications, pp. 185–201. Birkhauser Verlag, Basel, Switzerland.

ATKINSON, D. A., AND M. G. NETTING. 1927. The distribution and habits of the massasauga. Bulletin of the Antivenin Institute of America 1:40–44.

AVISE, J. C. 1994. Molecular Markers, Natural History and Evolution. Chapman and Hall, New York. 511 pp.

BAHRE, C. M. 1991. A Legacy of Change: Historic Human Impact on Vegetation of the Arizona Borderlands. University of Arizona Press, Tucson.

BAHRE, C. M. 1995. Human impacts on the grasslands of southeastern Arizona. *In* M. P. McClaran and T. R. Van Devender (eds.), The Desert Grassland, pp. 230–264. University of Arizona Press, Tucson.

BALLINGER, R. E. 1973. Comparative demography of two viviparous iguanid lizards (*Sceloporus jarrovi* and *Sceloporus poinsetti*). Ecology 54:269–283.

BALLINGER, R. E. 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi*, along an altitudinal gradient in southeastern Arizona. Ecology 60:901–909.

BALTOSSER, W. H., AND J. P. HUBBARD. 1985. New Mexico Ridgenose Rattlesnake Recovery Plan. U. S. Fish and Wildlife Service, Albuquerque, NM.

BAMFORD, M. 1985. The fire related dynamics of small vertebrates in *Banksia* woodland: a summary of research in progress. *In* J. Ford (ed.), Symposium on Fire Ecology and Management in Western Australian Ecosystems, pp. 107–110. W.A.I.T. Environmental Studies Group Publication No. 14, Western Australian Institute of Technology, Western Australia.

BARKER, D. G. 1991. An investigation of the natural history of the New Mexico ridgenose rattlesnake, *Crotalus willardi obscurus*. Unpubl. report to the New Mexico Department of Game and Fish Endangered Species Program. Santa Fe, NM.

BARKER, D. G. 1992. Variation, infrapecific relationships and biogeography of the ridgenose rattlesnake, *Crotalus willardi*. *In* J. A. Campbell and E. D. Brodie Jr. (eds.), Biology of the Pitvipers, pp. 89–106. Selva, Tyler, TX.

BARTLETT, R. D., AND A. TENANT. 2000. Snakes of North America: Western Region. Gulf Publishing Company, Houston, TX.

BARTON, N. H., AND M. SLATKIN. 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity 56:409–415.

BEAUPRE, S. J. 1995. Comparative ecology of the mottled rock rattlesnake, *Crotalus lepidus*, in Big Bend National Park. Herpetologica 51:45–56.

BEST, I. B. 1978. Field sparrow reproductive success and nesting ecology. Auk 95:9–22.

BETANCOURT, J. L., T. R. VAN DEVENDER, AND P. S. MARTIN (EDS.). 1990. Packrat middens: the last 40,000 years of biotic change. University of Arizona Press, Tucson.

BIELEMA, B. J. 1973. The Eastern massasauga (*Sistrurus catenatus catenatus*) in West-central Illinois. Unpublished Masters thesis. Western Illinois University, Macomb.

BIGALKE, R. C., AND K. WILLAN. 1984. Effects of fire regime on faunal composition and dynamics. *In* P. de V Booysen and N. M. Tainton (eds.), Ecological Effects of Fire in South African Ecosystems, pp. 255–271. Springer-Verlag, New York.

BLEM, C. R. 1982. Biennial reproduction in snakes: an alternative hypothesis. Copeia 1982:961–963.

BOCK, C. E., AND J. H. BOCK. 1978. Response of birds, small mammals and vegetation to burning sacaton grasslands in southeastern Arizona. Journal of Rangeland Management 31:296–300.

BOCK, C. E., H. M. SMITH, AND J. H. BOCK. 1990. The effect of livestock grazing upon abundance of the lizard *Sceloporus scalaris*, in southeastern Arizona. Journal of Herpetology 24: 445–446.

BOGERT, C. M., AND W. G. DEGENHARDT. 1961. An addition to the fauna of the United States, the Chihuahua ridge-nosed rattlesnake in New Mexico. American Museum Novitates 2064:1–15.

BRAITHWAITE, R. W. 1987. Effects of fire regimes on lizards in the wet-dry tropics of Australia. Journal of Tropical Ecology 3:265–275.

BROWN, D. E., AND C. H. LOWE. 1994. Biotic communities of the Southwest. A supplementary map to "Biotic communities: southwestern United States and northwestern Mexico. Brown, DE (ed.). University of Utah Press, Salt Lake City.

BRUSH, S. W., AND G. W. FERGUSON. 1986. Predation of lark sparrow eggs by a massasauga rattlesnake. Southwestern Naturalist 31:260–261.

BURNS, E. L. AND B. A. HOULDEN. 1999. Isolation and characterisation of microsatellite markers in the broad-headed snake (*Hoplocephalus bungaroides*). Molecular Ecology 8:520–521.

BUSHAR, L. M., H. K. REINERT, AND L. GELBERT. 1998. Genetic variation and gene flow within and between local populations of the timber rattlesnake, *Crotalus horridus*. Copeia 1998:411–422.

BUSHAR, L. M., M. MALIGA, AND H. K. REINERT. 2001. Cross-species amplification of *Crotalus horridus* microsatellites and their application in phylogenetic analysis. Journal of Herpetology 35:532–537.

CABIN, R. J., AND R. J. MITCHELL. 2000. To Bonferroni or not to Bonferroni: When and how are the questions. Bulletin of the Ecological Society of America 81:246–248.

CAMPBELL, J. A., AND W. W. LAMAR. 1989. The Venomous Reptiles of Latin America. Comstock Publ. Assoc., Ithaca, New York.

CAMPBELL, J. A., E. D. J. BRODIE, D. G. BARKER, AND A. H. PRICE. 1989. An apparent natural hybrid rattlesnake and *Crotalus willardi* (Viperidae) from the Peloncillo Mountains of southwestern New Mexico. Herpetologica 45:344–349.

CAPRIO, A. C., AND M. J. ZWOLINSKI. 1994. Fire and vegetation in a Madrean oak woodland, Santa Catalina Mountains, southeastern Arizona. *In* L. F. DeBano, G. J. Gottfried, R. H. Hamre, C. B. Edminster, P. F. Ffolliot, and A. Ortega-Rubio, (tech. coords.), Biodiversity and Management of the Madrean Archipelago: The Sky

Islands of Southwestern United States and Northwestern Mexico, pp. 389–398. USDA, Forest Service, General Technical Report RM–GTR–264, Fort Collins, CO.

CHANDLER, C. R., AND P. J. TOLSON. 1990. Habitat use by a boid snake, *Epicrates monensis*, and its anoline prey, *Anolis cristatellus*. Journal of Herpetology 24:151–157.

CHISZAR, D., C. W. RADCLIFFE, AND K. M. SCUDDER. 1977. Analysis of the behavioral sequence emitted by rattlesnakes during feeding episodes. I. Striking and chemosensory searching. Behavioral Biology [Behavioral and Neural Biology] 21:418–425.

COCK BUNING, T. DE. 1983. Thermal sensitivity as a specialization for prey capture and feeding in snakes. American Zoologist 23:363-375.

CONANT, R. 1951. The reptiles of Ohio. 2nd edition. American Midland Naturalist 20:1–284.

CONANT, R., AND J. T. COLLINS. 1991. A Field Guide to Reptiles and Amphibians. Eastern and Central North America. Houghton Mifflin Co., Boston, MA.

COOK, J. A. 1986. The mammals of the Animas Mountains and adjacent areas, Hidalgo County, New Mexico. Occasional Papers of the Museum of Southwestern Biology 4:1–45.

CORNUET, J.M., AND G. LUIKART. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001–2014.

CRAWFORD, R. 1936. A Study of the Massasauga as Found in Trumbull County, Ohio. Unpublished Masters thesis. University of Southern California, Los Angeles.

CURRAN, C. H. 1935. Rattlesnakes. Natural History 36:331–340.

CURTIN, C. G. 1997. Biophysical analysis of the impact of shifting land use on ornate box turtles, Wisconsin, USA. *In* J. Van Abbema, (ed.), Conservation, Restoration, and Management of Tortoises and Turtles, pp. 31–36. The New York Turtle and Tortoise Society and Wildlife Conservation Society Turtle Recovery Program, New York.

DARWIN, C. R. 1859. On the Origin of Species by Means of Natural Selection. Murray, London.

DARWIN, C. R. 1906. The Voyage of the Beagle. J. M. Dent and Sons, New York.

DEGENHARDT, W. G., C. W. PAINTER, AND A. H. PRICE. 1996. Amphibians and Reptiles of New Mexico. University of New Mexico Press, Albuquerque.

DELGADILLO ESPINOSA, J. A., E. GODINEZ CANO, F. CORREA SANCHEZ, AND A. GONZALEZ RUIZ. 1999. *Crotalus willardi silus* (Ridge-nosed Rattlesnake). Reproduction. Herpetological Review 30:168–169.

DI RIENZO, A., A. C. PETERSON, J. C. GARZA, A. M. VALDES, M. SLATKIN, AND N. B. FREIMER. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proceeding of the National Academy of Sciences (USA) 91:3166–3170.

DILLER, L. V., AND R. L. WALLACE. 1984. Reproductive biology of the northern Pacific rattlesnake (*Crotalus viridis oreganus*) in northern Idaho. Herpetologica 40:182–193.

DITMARS, R. L. 1907. The Reptile Book. Doubleday, Page, and Company, New York.

DITMARS, R. L. 1912. The feeding habits of serpents. Zoologica 1:197–238.

DODD JR., C. K. 1987. Status, conservation, and management. *In* R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), Snakes: Ecology and Evolutionary Biology, pp. 478-513. McGraw-Hill, New York, NY.

DOWNES, S., AND R. SHINE. 1998. Sedentary snakes and gullible geckos: Predator-prey coevolution in nocturnal rock-dwelling reptiles. Animal Behavior 55:1373–1385.

DUVALL, D., D. CHISZAR, W. K. HAYES, J. K. LEONHARDT, AND M. J. GOODE. 1990a. Chemical and behavioral ecology of foraging in prairie rattlesnakes (*Crotalus viridis viridis*). Journal of Chemical Ecology 16:87–101.

DUVALL, D., M. J. GOODE, W. K. HAYES, J. K. LEONHARDT, AND D. G. BROWN. 1990b. Prairie rattlesnake vernal migration: field experimental analyses and survival value. National Geographic Research 1:80–111.

DUVALL, D., S. J. ARNOLD, AND G. W. SCHUETT. 1992. Pitviper mating systems: Ecological potential, sexual selection, and microevolution. In J. A. Campbell and E. D. Brodie, Jr. (eds.), Biology of the Pitvipers, pp. 321–336. Selva, Tyler, TX

EBERLY, L. D., AND T. B. STANLEY. 1978. Cenozoic stratigraphy and geologic history of southwestern Arizona. Geological Society of America Bulletin 89: 921–940.

ERNST, C. H. 1992. Venomous Reptiles of North America. Smithsonian Institute Press, Washington, DC.

ERWIN, W. J., AND R. H. STASIAK. 1979. Vertebrate mortality during the burning of a reestablished prairie in Nebraska. American Midland Naturalist 101:247–249.

EVERMANN, B. W., AND H. W. CLARK. 1914. The snakes of the Lake Maxinkuckee Region. Proceedings of the Indiana Academy of Sciences 24:337–348.

FELSENSTEIN, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c.

FITCH, H. S. 1960. Autecology of the copperhead. University of Kansas Publications of the Museum of Natural History 13:85–288.

FITCH, H. S. 1970. Reproductive cycles in lizards and snakes. University of Kansas Publications of the Museum of Natural History Miscellaneous Publications 52:1–247.

FITCH, H. S. 1985. Variation in clutch and litter size in New World reptiles. University of Kansas Museum of Natural History Miscellaneous Publications 76:1–76.

FITCH, H. S., AND G. R. PISANI. 1993. Life history traits of the western diamondback rattlesnake (*Crotalus atrox*) studied from roundup samples in Oklahoma. Occasional Papers of the Museum of Natural History, University of Kansas 156:1–24.

FITZSIMMONS, N. N., C. MORITZ, AND S. S. MOORE. 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Molecular Biology and Evolution 12:432–440.

FORSMAN, A., AND R. SHINE. 1997. Rejection of non-adaptive hypotheses for intraspecific variation in trophic morphology in gape-limited predators. Biological Journal of the Linnean Society 62:209–223.

FOWLIE, J. A. 1965. The Snakes of Arizona. Azul Quinta Press, Fallbrook, CA.

FOX, W. S. 1948. The serpent in the garden. Queen's Quarterly 55:58–66.

FRIEND, G. R. 1993. Impact of fire on small vertebrates in Mallee woodlands and heathlands of temperate Australia. Biological Conservation 65:99–114.

GARZA, J. C., AND E. WILLIAMSON. 2001. Detection of reduction in population size using data from tandemly repeated loci. Molecular Ecology 10:305–318.

GAUTSCHI B., A. WIDMER, AND J. KOELLA. 2000. Isolation and characterization of microsatellite loci in the dice snake *(Natrix tesselata)*. Molecular Ecology 9:2191–2193.

GIBBS, H. L., K. PRIOR, AND C. PARENT. 1998. Characterization of DNA microsatellite loci from a threatened snake: The eastern massasauga rattlesnake (*Sistrurus c. catenatus*) and their use in population studies. Journal of Heredity 89:169–173.

GIBBS, H. L., K. A. PRIOR, AND P. J. WEATHERHEAD. 1994. Genetic analysis of populations of threatened snake species using RAPD markers. Molecular Ecology 1994:329–337.

GIBBS, H. L., K. A. PRIOR, P. J. WEATHERHEAD, AND G. JOHNSON. 1997. Genetic structure of populations of the threatened eastern massasauga rattlesnake, *Sistrurus c.catenatus*: evidence from microsatellite DNA markers. Molecular Ecology 6:1123–1132.

GODLEY, J. S. 1980. Foraging ecology of the striped swamp snake, *Regina alleni*, in southern Florida. Ecological Monographs 50:411–436.

GOLDBERG, S. R. 1999a. Reproduction in the tiger rattlesnake, *Crotalus tigris* (Serpentes: Viperidae). Texas Journal of Science 51:31–36.

GOLDBERG, S. R. 1999b. Reproduction in the blacktail rattlesnake, *Crotalus molossus* (Serpentes: Viperidae). Texas Journal of Science 51:323–328.

GOLDBERG, S. R. 2000. Reproduction in the twin-spotted rattlesnake, *Crotalus pricei* (Serpentes: Viperidae). Western North American Naturalist 60:98–100.

GOLDBERG, S. R., AND A. T. HOLYROSS. 1999. Reproduction in the desert massasauga, *Sistrurus catenatus edwardsii*, in Arizona and Colorado. Southwestern Naturalist 44:531–535.

GOLDBERG, S. R., AND W. S. PARKER. 1975. Seasonal testicular histology of the colubrid snakes, *Masticophis taeniatus* and *Pituophis melanoleucus*. Herpetologica 31:317–322.

GOLDSTEIN, D. B., A. R. LINARES, L. L. CAVALLI-SFORZA, AND M. W. FELDMAN. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463–471.

GOTELLI, N. J. 1995. A Primer of Ecology. Sinauer Assoc., Sunderland, MA.

GOUDET, J. 1995. FSTAT version 2.9: a computer program to calculate F-statistics. Journal of Heredity 86:485–486.

GREENBERG, C. H., D. G. NEARY, AND L. D. HARRIS. 1994. Effect of high-intensity wildfire and silvicultural treatments on reptile communities in sand-pine scrub. Conservation Biology 8:1047–1057.

GREENE, H. W. 1983. Dietary correlates of the origin and radiation of snakes. American Zoologist 23:431–441.

GREENE, H. W. 1992. The ecological and behavioral context for pitviper evolution. *In* J. A. Campbell and E. D. Brodie, Jr. (eds.), Biology of the Pitvipers, pp. 107–117. Selva, Tyler, TX.

GREENE, H. W. 1994. Systematics and natural history: Foundations for understanding and conserving biodiversity. American Zoologist 34:48–56.

GREENE, H. W. 1997. Snakes: The Evolution of Mystery in Nature. University California Press, Berkeley.

GREENE, H. W., AND J. A. CAMBPELL. 1992. The future of pitvipers. *In* J. A. Campbell and E. D. Brodie Jr. (eds.), Biology of the Pitvipers, pp. 421–427. Selva, Tyler, Texas.

GREENE, H. W., AND G. V. OLIVER JR. 1965. Notes on the natural history of the western massasauga. Herpetologica 21:225–228.

GUTIÉRREZ-ESPELETA, G. A., S. T. KALINOWSKI, W. M. BOYCE, AND P. W. HEDRICK. 2000. Genetic variation and population structure in desert bighorn sheep: implications for conservation *Conservation Genetics* 1: 3–15.

HALLOCK, L. A. 1991. Habitat Utilization, Diet and Behavior of the Eastern Massasauga (*Sistrurus catenatus*) in Southern Michigan. Unpublished Master's thesis, Michigan State University, East Lansing.

HAMILTON, W. J., JR., AND J. A. POLLACK. 1955. The food of some crotalid snakes from Fort Benning, Georgia. Natural History Miscellanea 140:1–4.

HAMMERSON, G. A. 1986. Amphibians and Reptiles in Colorado. Colorado Division of Wildlife, Denver.

HAMMERSON, G. A. 1999. Amphibians and Reptiles in Colorado. 2nd edition. University Press of Colorado, Niwot.

HARRIS, H. S., AND R. S. SIMMONS. 1976. The paleogeography and evolution of *Crotalus willardi*, with a formal description of a new subspecies from New Mexico, United States. Bulletin of the Maryland Herpetology Society 12:1–22.

HARRIS, H. S., JR., AND R. S. SIMMONS. 1975. An endangered species, the New Mexican ridge-nosed rattlesnake. Bulletin of the Maryland Herpetological Society 11:1–7.

HASTINGS, J. R. 1959. Vegetation change and arroyo cutting in southeastern. Journal of the Arizona Academy of Science 1: 60–66.

HAYNES, C. V. 1991. Geoarchaeological and paleohydrological evidence for a Clovis-age drought in North America and its bearing on extinction. Quaternary Research 35:438–450.

HEATWOLE, H., AND E. DAVISON. 1976. A review of caudal luring in snakes with notes on its occurrence in the Saharan sand viper, *Cerastes vipera*. Herpetologica 32:332–226.

HEDRICK, P. W. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318.

HEDRICK, P. W., K. M. PARKER, AND R. N. LEE. 2001. Using microsatellite and MHC variation to identify species, ESUs, and MUs in the endangered Sonoran topminnow. Molecular Ecology 10:1399–1412.

HOBERT, J. P. 1997. The massasauga rattlesnake (*Sistrurus catenatus*) in Colorado. Unpublished Master's thesis, University of Northern Colorado, Greeley.

HOLBROOK, S. J. 1979. Vegetational affinities, arboreal activity, and coexistence of three species of rodents. Journal of Mammalogy 60:528–542.

HOLMAN, J. A. 2000. Fossil Snakes of North America: Origin, Evolution, Distribution, Paleoecology. Indiana University Press, Bloomington.

HOLYCROSS, A. T. 2000. *Crotalus willardi obscurus*. Caudal dichromatism. Herpetological Review 31:246.

HOLYCROSS, A. T., AND S. R. GOLDBERG. 2001. Reproduction in northern populations of the ridgenose rattlesnake, *Crotalus willardi* (Serpentes: Viperidae). Copeia 2001:473–481.

HOLYCROSS, A. T., C. W. PAINTER, D. G. BARKER, AND M. E. DOUGLAS. *In press.* Foraging ecology of a threatened rattlesnake, *Crotalus willardi obscurus*. *In* Biology of the Vipers, G. W. Schuett, M. Hoggren M. E. Douglas and H. W. Greene (eds.). Biological Sciences Press, Carmel, Indiana.

HOLYCROSS, A. T., M. E. DOUGLAS, J. R. HIGBEE, AND R. H. BOGDEN. In prep. Isolation and Characterization of Microsatellite Loci from a Threatened Rattlesnake (New Mexico Ridgenose Rattlesnake, *Crotalus willardi obscurus*).

HUMPHREY, R. R. 1958. The desert grassland. A history of vegetational change and an analysis of causes. The Botanical Review XXIV:193–252.

HUMPHREY, R. R. 1987. 90 Years and 535 Miles. University of New Mexico Press, Albuquerque, NM.

JAMESON, E. W., JR. 1952. Food of deer mice, *Peromyscus maniculatus* and *P. boylii*, in the northern Sierra Nevada, California. Journal of Mammalogy 33:50–60.

JEMISON, S. C., L. A. BISHOP, P. G. MAY, AND T. M. FARRELL. 1995. The impact of PIT-tags on growth and movement of the rattlesnake *Sistrurus miliarus*. Journal of Herpetology 29:129–132.

JOHNSON, G. 1995. Spatial Ecology, Habitat Preference, and Habitat Management of the Eastern Massasauga, *Sistrurus c. catenatus*, in a New York Weakly-minerotrophic Peatland. Unpubl. Ph.D. dissertation, State University of New York, Syracuse.

JOHNSON, G. 2000. Spatial ecology of the eastern massasauga (*Sistrurus c. catenatus*) in a New York peatland. Journal of Herpetology 34:186–192.

JOHNSON, G., AND D. J. LEOPOLD. 1998. Habitat management for the eastern massasauga in a central New York peatland. Journal of Wildlife Management 62:84–97.

JOHNSON, L. F., J. S. JACOB, AND P. TORRANCE. 1982. Annual testicular and androgenic cycles of the cottonmouth (*Agkistrodon piscivorus)* in Alabama. Herpetologica 38:16–25.

KAIB, M., C. H. BAISAN, H. D. GRISSINO-MAYER, AND T. W. SWETNAM. 1996. Fire history in the gallery pine-oak forests and adjacent grasslands of the Chiricahua Mountains of Arizona. *In* P. F. Ffolliot, M. B. Baker, G. J. Gottfried, G. Solis-Garza, C. B. Edminster, D. G. Neary, L. S. Allen, and R. H. Hamre (tech. coords.), Effects of Fire on Madrean Province Ecosystems, pp. 253–264. USDA Forest Service, General Technical Report RM–GTR–298, Fort Collins, CO.

KAUFFELD, C. F. 1943. Field notes on some Arizona reptiles and amphibians. American Midland Naturalist 29:342–359.

KEENLYNE, K. D. 1968. The Massasauga (*Sistrurus catenatus catenatus*) in Buffalo County, Wisconsin. Unpubl. Master's thesis. University of Minnesota, Minneapolis.

KEENLYNE, K. D. 1978. Reproductive cycles in two species of rattlesnakes. American Midland Naturalist 100:368–375.

KEENLYNE, K. D., AND J. R. BEER. 1973. Food habits of *Sistrurus catenatus catenatus*. Journal of Herpetology 7:381–382.

KING, R. B. 2000. Analyzing the relationship between clutch size and female body size in reptiles. Journal of Herpetology 34:148–150.

KLAUBER, L. M. 1949. The subspecies of the ridge-nosed rattlesnake, *Crotalus willardi*. Transactions of the San Diego Society of Natural History 11:121–140.

KLAUBER, L. M. 1956. Rattlesnakes: their habits, life histories, and influence on mankind. Volume I. University of California Press, Berkeley.

KLAUBER, L. M. 1972. Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind. 2nd edition. University of California Press, Berkeley.

KRUSE, W. H., G. J. GOTTFRIED, D. A. BENNETT, AND H. MATA-MANQUEROS. 1996. The role of fire in Madrean encinal oak and pinyon-juniper woodland development. *In* P. F. Ffolliot, P. F., M. B. Baker, G. J. Gottfried, G. Solis-Garza, C. B. Edminster, D. G. Neary, L. S. Allen, and R. H. Hamre (tech. coords.), Effects of Fire on Madrean Province Ecosystems, pp. 99–106. USDA Forest Service, General Technical Report RM–GTR–298, Fort Collins, CO.

LANDE, R. 1988. Genetics and demography in biological conservation. Science241:1455–1460.

LARDIE, R. L. 1976. Large centipede eaten by a western massasauga. Bulletin of the Oklahoma Herpetological Society 1:40.

LEOPOLD, A. S. 1924. Grass, brush, timber and fire in southern Arizona. Journal of Forestry 22:1–10.

LERAY, W. J. 1930. The rattlesnake (*Sistrurus catenatus*) in Ontario. Canadian Field-Naturalist 44:201–203.

LEVENE, H. 1949. On a matching problem in genetics. Ann. Math Stat. 20:91–94.

LEVITON, A. E., R. H. GIBBS JR., E. HEAL, AND C. E. DAWSON. 1985. Standards in herpetology and ichthyology. Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.

LILLYWHITE, H. B., AND E. NORTH. 1974. Perching behavior of *Sceloporus occidentalis* in recently burned chaparral. Copeia 1974:256–257.

LOEWEN, S. L. 1947. Notes on a rattlesnake in captivity. Turtox News 25:53–54.

LOUGHEED, S. C., H. L. GIBBS, K. A. PRIOR, AND P. J. WEATHERHEAD. 1999. Hierarchical patterns of genetic population structure in black rat snakes (*Elaphe obsoleta obsoleta*) as revealed by microsatellite DNA analysis. Evolution 53:1995–2001.

LOWE, C. H. 1967. The Vertebrates of Arizona. University of Arizona Press, Tucson.

LOWE, C. H., C. R. SCHWALBE, AND T. B. JOHNSON. 1986. The Venomous Reptiles of Arizona. Arizona Game and Fish Department, Phoenix.

LYON, M. W., JR., AND C. A. BISHOP. 1936. Bite of the prairie rattlesnake, *Sistrurus catenatus* Raf. Proceedings of the Indiana Academy of Sciences 45:253–256.

MACKESSY, S. P. 1988. Venom ontogeny in the Pacific rattlesnakes *Crotalus viridis helleri* and *C. v. oreganus*. Copeia 1988:92–101.

MADSEN, T., AND R. SHINE. 1996. Seasonal migration of predators and prey - a study of pythons and rats in tropical Australia. Ecology 77:149–156.

MANION, S. 1968. *Crotalus willardi* –the Arizona ridge-nosed rattlesnake. Herpetology 2:27–30.

MARSHALL, J. T. 1957. Birds of pine-oak woodland in southern Arizona and adjacent Mexico. Pacific Coast Avifauna 32:1–125.

MARSHALL, T. C., J. SLATE, L. E. B. KRUUCK, AND J. M. PEMBERTON.1998. Statistical confidence for likely-hood based paternity inference in natural populations. Molecular Ecology 7:639–655.

MARTIN, B. E. 1975a. A brood of Arizona ridge-nosed rattlesnakes (*Crotalus willardi willardi*) bred and born in captivity. Bulletin of the Maryland Herpetological Society 11:187–189.

MARTIN, B. E. 1975b. Notes on a brood of the Arizona ridge-nosed rattlesnake *Crotalus willardi willardi*. Bulletin of the Maryland Herpetological Society 11:64–65.

MARTIN, B. E. 1975c. An occurrence of the Arizona ridge-nosed rattlesnake, *Crotalus willardi willardi*, observed feeding in nature. Bulletin of the Maryland Herpetological Society 11:66–67.

MARTIN, B. E. 1976. A reproductive record for the New Mexican ridge-nosed rattlesnake (*Crotalus willardi obscurus).* Bulletin of the Maryland Herpetological Society 12:126–128.

MARTIN, P. S. 1963a. Geochronology of pluvial Lake Cochise, southern Arizona. II Pollen analysis of a 42-meter core. Ecology 44:436–445.

MARTIN, P. S. 1963b. The last 10,000 years - a fossil pollen record of the American Southwest. University of Arizona Press, Tucson.

MARTIN, P. S., AND J. E. MOSIMAN. 1965. Geochronology of pluvial Lake Cochise, southern Arizona, III. Pollen statistics and Pleistocen metastability. American Journal of Science 263:313–358.

MASLIN, T. P. 1965. The status of the rattlesnake *Sistrurus catenatus* (Crotalidae) in Colorado. Southwestern Naturalist 10:31–34.

MAUGER, D., AND T. P. WILSON. 1999. Population characteristics and seasonal activity of *Sistrurus catenatus catenatus* in Will County, Illinois: Implications for management and monitoring. *In* B. Johnson and M. Wright (eds.), Second International Symposium and Workshop on the Conservation of the Eastern Massasauga Rattlesnake, *Sistrurus catenatus catenatus*: Population and Habitat Management Issues in Urban, Bog, Prairie and Forested ecosystems, pp. 110–124. Toronto Zoo, Toronto, Ontario.

MCCRACKEN, G.F., G. M. BURGHARDT, AND S. E. HOUTS.1999. Microsatellite markers and multiple paternity in the garter snake *Thamnophis sirtalis*. Molecular Ecology 8:1475–1479.

MCCRANIE, J. R., AND L. D. WILSON. 1987. The biogeography of the herpetofauna of the pine-oak woodlands of the Sierra Madre Occidental of Mexico. Milwaukee Public Museum Contributions in Biology and Geology 72:1–30.

MCKINNEY, C. O., AND R. E. BALLINGER. 1966. Snake predators of lizards in western Texas. Southwestern Naturalist 11:410–412.

MCPHERSON, G. R., T. W. BOUTTON, AND A. J. MIDWOOD. 1993. Stable carbon isotope analysis of soil organic matter illustrates vegetation change at the grassland/woodland boundary in southeastern Arizona, USA. Oecologia 93:95–101.

MEAD, J. I. 1975. The 1975 Field Season Faunal Remains from the Lehner Site, Arizona. Report submitted to C. V. Haynes, Dept of Anthropology, University of Arizona, Tucson.

MEFFE, G. K., AND C. R. CARROLL, (EDS.). 1994. Priniciples of Conservation Biology. Sinauer Associates, Inc., Sunderland, Massachusetts.

MENGES, C. M., AND P. A. PEARTHREE. 1989. Late Cenozoic tectonism in Arizona and its impact on regional landscape evolution. *In*: J. P. Jenney and S. J. Reynolds (eds.), Geologic Evolution of Arizona, pp. 649–680. Arizona Geological Society Digest 17, Tucson, Arizona.

MINTON, S. A. 1972. Amphibians and Reptiles of Indiana. Indiana Acadamy of Science Monograph No. 3.

MINTON, S. A. 1983. *Sistrurus catenatus* (Rafinesque Massasauga. Catalogue of American Amphibians and Reptiles:332.1–332.2.

MOORE, S.S., L. L. SARGEANT, T. J. KING, J. S. MATTICK, M. GEORGES, AND D. J. S. HETZEL. 1991. The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10:654–660.

MOORE, T. D., L. E. SPENCE, C.E. DUGNOLLE, AND W. G. HEPWORTH. 1974. Identification of the Dorsal Guard Hairs of Some Mammals of Wyoming. Wyoming Game and Fish Department, Cheyenne.

MORITZ, C. 1994. Defining 'Evolutionarily Significant Units" for conservation. Trends in Ecology and Evolution 9:373–374.

MUSHINSKY, H. R. 1985. Fire and the Florida sandhill herpetofaunal community: with special attention to the responses of *Cnemidophorus sexlineatus*. Herpetologica 41:333–342.

MUSHINSKY, H. R. 1987. Foraging ecology. *In* R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), Snakes: Ecology and Evolutionary Biology, pp. 302–334. McGraw-Hill, New York, NY.

MUSHINSKY, H. R. 1992. Natural history and abundance of southeastern five-lined skinks, *Eumeces inexpectatus*, on a periodically burned sandhill in Florida. Herpetologica 48:307–312.

NEI, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

NETTING, M. G. 1932. The Poisonous Snakes of Pennsylvania. Carnegie Museum of Vertebrate Zoology Pamphlet No. 1.

PARK, S. D. E. 2001. Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. Unpubl. Ph.D. dissertation. University of Dublin, Ireland.

PARKER, K. W., AND S. C. MARTIN. 1952. The mesquite problem on southern Arizona ranges. U.S. Department of Agriculture Circular 908.

PARKER, S. A., AND D. STOTZ. 1977. An observation on the foraging behavior of the Arizona ridge-nosed rattlesnake, *Crotalus willardi willardi* (Serpentes: Crotalidae). Bulletin of the Maryland Herpetological Society 13:123.

PARKER, W. S., AND M. V. PLUMMER. 1987. Population ecology. *In* R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), Snakes: Ecology and Evolutionary Biology, pp. 253-301. McGraw-Hill, New York, NY.

PIANKA, E. R. 1989. Desert lizard diversity: additional comments and some data. American Naturalist 134:344–364.

POPE, T. E. B. 1926. The massasauga in Wisconsin. Yearbook of the Public Museum, City of Milwaukee 1:171–180.

PRIMMER, C. R., A. P. MOLLER, AND H. ELLEGREN. 1996. Wide-ranging survey of cross-species microsatellite amplification in birds. Molecular Ecology 5:365–378.

PRIOR, K., H. L. GIBBS, AND P. J. WEATHERHEAD. 1997. Population genetic structure in the black rat snake: Implications for management. Conservation Biology 11:1147–1158.

PROSSER, M. R., H. L. GIBBS, AND P. J. WEATHERHEAD. 1999. Microgeographic population genetic structure in the northern water snake, *Nerodia sipedon sipedon* detected using microsatellite DNA loci. Molecular Ecology 8:329–333.

QUELLAR, D. C., J. E. STRASSMANN, AND C. R. HUGHES. 1993. Microsatellites and Kinship. Trends in Ecology and Evolution 8:285–288.

QUINN, H. R. 1977. Further notes on reproduction in *Crotalus willardi* (Reptilia, Serpentes, Crotalidae). Bulletin of the Maryland Herpetological Society 13:111.

QUINN, R. D. 1994. Animals, fire, and vertebrate herbivory in Californian Chaparral and other Mediterranean-type ecosystems. In J. M. Moreno, W. C. Oechel (eds.), The Role of Fire in Mediterranean-type Ecosystems, pp. 46–78. Springer-Verlag, New York.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

REINERT, H. K. 1978. The Ecology and Morphological Variation of the Massasauga Rattlesnake, *Sistrurus catenatus*. Unpubl. Masters thesis. Pennsylvania State University, Clarion.

REINERT, H. K. 1981. Reproduction by the massasauga (*Sistrurus catenatus catenatus*). American Midland Naturalist 105:393–395.

REINERT, H. K., AND D. CUNDALL. 1982. An improved surgical implantation method for radio-tracking snakes. Copeia 1982:702–705.

REINERT, H. K., AND W. R. KODRICH. 1982. Movements and habitat utilization by the massasauga, *Sistrurus catenatus catenatus*. Journal of Herpetology 16:162–171.

REINERT, H. K., D. CUNDALL, AND L. M. BUSHAR. 1984. Foraging behavior of the timber rattlesnake, *Crotalus horridus*. Copeia 1984:976–981.

REISERER, R. S. 2001. Evolution of Life Histories in Rattlesnakes. Unpublished Ph. D. dissertation. University of California, Berkeley.

REISERER, R. S. *In press*. Stimulus control of caudal luring and other feeding responses: A program for research on visual perception in vipers. *In* G. W. Schuett, M. Hoggren, M. E. Douglas, and H. W. Greene (eds.), Biology of the Vipers. Biological Sciences Press, Carmel, Indiana.

REYNOLDS, R. P., AND N. J. SCOTT, JR. 1982. Use of a Mammalian Resource by a Chihuahuan Snake Community. *In* N. J. Scott Jr. (ed.), Herpetological Communities, pp. 99–118. U. S. Fish and Wildlife Service, Washington, DC.

RODRÍGUEZ-ROBLES, J. A., AND H. W. GREENE. 1996. Ecological patterns in Greater Antillean macrostomatan snake assemblages, with comments on body-size evolution in Epicrates (Boidae). *In* R. Powell and R. W. Henderson (eds.), Contributions to West Indian Herpetology: A Tribute to Albert Schwartz, pp. 339–357. Society for the Study of Amphibians and Reptiles, Ithaca, NY.

RODRÍGUEZ-ROBLES, J. A., D. G. MULCAHY, AND H. W. GREENE. 1999. Feeding ecology of the desert nightsnake, *Hypsiglena torquata* (Colubridae). Copeia 1999:93–100.

ROHLF, F. J., AND D. E. SLICE. 1999. BIOMstat for Windows: Statistical software for biologists. Version 3.3. Exeter Software, New York, NY.

ROSSI, J. V., AND J. J. FELDNER. 1993. *Crotalus willardi willardi* and *Crotalus lepidus klauberi*: Arboreal behavior. Herpetological Review 24:35.

ROTH, E. D., P. G. MAY, AND T. M. FARRELL. 1999. Pigmy rattlesnakes use frog-derived chemical cues to select foraging sites. Copeia 1999:772–774.

RUBIO, M. 1998. Rattlesnake: Portrait of a Predator. Smithsonian Institution Press, Washington, DC.

RUTHVEN, A. G. 1911. A biological survey of the sand dune region on the south shore of Saginaw Bay. Michigan Geol. Biol. Survey Publ. 4 (Series 2):270–271.

RUTHVEN, A. G., C. THOMPSON, AND H. T. GAIGE. 1928. The Herpetology of Michigan. University of Michigan Museum, Michigan Handbook Series (3).

SAINT GIRONS, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. Herpetologica 38:5–16.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SCARBOROUGH, R. B., AND H. W. PIERCE. 1978. Late Cenozoic basins in Arizona. *In* J. F. Callender, J. C. Wilt, and R. E. Clemons (Eds.), Land of Cochise, pp. 253–259. New Mexico Geological Society Guidebook, 29th Field Conference, Socorro, New Mexico.

SCHAEFER, W. H. 1934. Diagnosis of sex in snakes. Copeia 1934:181.

SCHMIDT, K. P. 1938. Herpetological evidence for the postglacial eastward extension of the steppe in North America. Ecology 19:396–497.

SCHUETT, G. W., D. L. CLARK, AND F. KRAUS. 1984. Feeding mimicry in the rattlesnake *Sistrurus catenatus*, with comments on the evolution of the rattle. Animal Behaviour 32:625–626.

SCHWAMMER, H. 1983. Herpetologische beobachtungen aus Colorado/USA. Aasfressen bei *Sistrurus catenatus edwardsi/tergeminus* und verhaltensmimikry bei *Pituophis melanoleucus s.* Aquaria 30:90–93.

SECOR, S. M. 1995. Ecological aspects of foraging mode for the snakes *Crotalus cerastes* and *Masticophis flagellum.* Herpetological Monographs 9:169–186.

SECOR, S. M., AND J. DIAMOND. 1998. A vertebrate model of extreme physiological regulation. Nature 395:659–662.

SEIGEL, R. A. 1984. The foraging ecology and resource partitioning patterns of two species of garter snakes. Unpublished Ph.D. dissertation. University of Kansas, Lawrence.

SEIGEL, R. A. 1986. Ecology and conservation of an endangered rattlesnake, *Sistrurus catenatus*, in Missouri, USA. Biological Conservation 35:333–346.

SEIGEL, R. A., AND N. B. FORD. 1987. Reproductive ecology. *In* R. A. Seigel, J. T. Collins, and S. S. Novak (eds.), Snakes: Ecology and Evolutionary Biology, pp. 210–252. Macmillan Publ. Co., New York.

SEIGEL, R. A., C. A. SHEIL, AND J. S. DOODY. 1998. Changes in a population of an endangered rattlesnake *Sistrurus catenatus* following a severe flood. Biological Conservation 83:127–131.

SELOUS, P. S. 1900. Notes and observations regarding the habits and characteristics of the massasauga or ground rattlesnake, *Sistrurus catenatus*, during captivity. Michigan Academy of Science First Report:89–92.

SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82–90.

SHINE, R. A. 1996. Life-history evolution in Australian snakes: A path analysis. Oecologia: 107:484–489.

SHINE, R. A., J. K. WEBB, M. FITZGERALD, AND J. SUMNER. 1998. The impact of bush-rock removal on an endangered snake species, *Hoplocephalus bungaroides* (Serpentes: Elapidae). Wildlife-Research 25:285–295.

SIMONS, L. H. 1989. Vertebrates killed by desert fire. Southwestern Naturalist 34:144.

SLATKIN, M. 1995. A Measure of Population Subdivision Based on Microsatellite Allele Frequencies. Genetics 139:457–462.

SMARTT, R. A. 1978. A comparison of ecological and morphological overlap in a *Peromyscus* community. Ecology 59:216–220.

SMITH, H. M., AND F. N. WHITE. 1955. Adrenal enlargement and its significance in the hognose snakes (*Heterodon*). Herpetologica 11:137–144.

SMITH, L. J., A. T. HOLYCROSS, C. W. PAINTER, AND M. E. DOUGLAS. 2001. Montane rattlesnakes and prescribed fire. Southwestern Naturalist 46:54–61.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry. 3rd edition. W. H. Freeman and Company, New York.

SPAULDING, W. G., AND L. J. GRAUMLICH. 1986. The last pluvial climatic episodes in the deserts of southwestern North America. Nature 320: 441–444.

STEBBINS, R. C. 1985. A Field Guide to Western Reptiles and Amphibians. 2nd edition. Houghton Mifflin, New York.

STRIMPLE, P. D. 1992. Caudal luring: A discussion on definition and application of the term. Greater Cincinnati Herpetological Society Contributions in Herpetology 1992:49–54.

STUWE, M., AND C. E. BLOHOWIAK. 1992. McPAAL: micro-computer programs for the analysis of animal locations. Manual for version 1.20 for DOS (with addendum for version 1.22, March 1992). Smithsonian Institution, Front Royal, VA.

SWANSON, P. L. 1930. Notes on the massasauga. Bulletin of the Antivenin Institute of America 4:70–71.

SWETNAM, T. W., AND C. H. BAISAN. 1994. Historical fire regime patterns in the southwestern United States since AD 1700. *In* C. D. Allen (ed.), Fire Effects in Southwestern Forests, pp. 11–32. USDA Forest Service, General Technical Report RM–GTR–286, Fort Collins, CO.

SWETNAM, T. W., AND C. H. BAISAN. 1996. Fire histories of montane forests in the Madrean Borderlands. *In* P. F. Ffolliot, M. B. Baker, G. J. Gottfried, G. Solis-Garza, C. B. Edminster, D. G. Neary, L. S. Allen, and R. H. Hamre (tech. coords), pp. 15–36. Effects of Fire on Madrean Province Ecosystems. USDA Forest Service, General Technical Report RM–GTR–298, Fort Collins, CO

TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463–6471.

TAYLOR, E. N. 2001. Diet of the Baja California Rattlesnake, *Crotalus enyo* (Viperidae). Copeia 2001:552–554.

TAYLOR, W. E. 1892. Catalogue of the snakes of Nebraska with notes on their habits and distribution. American Naturalist 26:742–752.

TRYON, B.W. 1978. Reproduction in a pair of captive Arizona ridge-nosed rattlesnakes, *Crotalus willardi willardi* (Reptilia, Serpentes, Crotalidae). Bulletin of the Maryland Herpetological Society 14:83–88.

UNITED STATES FISH AND WILDLIFE SERVICE. 1978. Listing of the New Mexican ridgenosed rattlesnake as a threatened species with critical habitat. Federal Register 43:34476–34480.

UNITED STATES FISH AND WILDLIFE SERVICE. 1988. Endangered Species Act of 1973. As Amended Through the 100th Congress. U.S. Department of the Interior, Washington, D.C.

UNITED STATES FOREST SERVICE. 1997. Environmental assessment: Maverick Prescribed Burn. Douglas Ranger District, Coronado National Forest, Douglas, AZ.

VALDES, A. M., M. SLATKIN, AND N. B. FREIMER. 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics 133:737–739.

VAN DENBURGH, J. 1922. The reptiles of western North America. Vol. II. Snakes and turtles. Occasional Papers of the California Academy of Sciences (10):617–1028.

VAN DEVENDER, R. R., AND J. I. MEAD. 1978. Early Holocene and Late Pleistocene amphibians and reptiles in Sonoran Desert packrat middens. Copeia 1978:464–475.

VAN DEVENDER, T. R. 1995. Desert Grassland History; Changing climates, evolution, biogeography, and community dynamics. *In*: M. P. McClaran and T. R. Van Devender (Eds.), The Desert Grassland, pp. 68–99. University of Arizona Press, Tucson.

VAN DEVENDER, T. R., A. M. REA, AND W. E. HALL. 1991. Faunal analysis of late quaternary vertebrates from Organ Pipe Cactus National Monument, southwestern Arizona. Southwestern Naturalist 36: 94–106.

VAN DEVENDER, T. R., AND R. D. WORTHINGTON. 1977. The herpetofauna of Howell's Ridge Cave and the paleoecology of the northwestern Chihuahuan Desert. *In* R. H. Wauer and D. H. Riskind (eds.), Transactions of the Symposium on the Biological Resources of the Chihuahuan Desert Region, United States and Mexico, pp. 85–106. National Park Service Transactions and Proceedings Serial No. 3, Washington, DC.

VAN DEVENDER, T. R., R. S. THOMPSON, AND J. L. BETANCOURT. 1987. Vegetation history of the deserts of southwestern North America: the nature and timing of the late Wisconsin-Holocene transition. *In* W. F. Ruddiman and H. E. Wright, Jr. (eds.), North America and Adjacent Oceans During the Last Deglaciation, pp. 323–352. Geological Society of America, Boulder, CO.

VILLARREAL, X., J. BRICKER, H. K. REINERT, L. GELBERT, AND L. M. BUSHAR. 1996. Isolation and characterization of microsatellite loci for use in population genetic analysis in the timber rattlesnake, *Crotalus horridus*. Journal of Heredity 87:152–155.

VITT, L. J. 1983. Ecology of an anuran-eating guild of terrestrial tropical snakes. Herpetologica 39:52–66.

VORHIES, C. T. 1948. Food items of rattlesnakes. Copeia 1948:302–303.

WAPLES, R. S. 1995. Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act. American Fisheries Society Symposium 17:8–27.

WEATHERHEAD, P. J., AND K. A. PRIOR. 1992. Preliminary observations of habitat use and movements of the eastern massasauga rattlesnake (*Sistrurus c. catenatus*). Journal of Herpetology 26:447–452.

WEBB, R. G. 1970. Reptiles of Oklahoma. University of Oklahoma Press, Norman.

WEIR, B. S. AND C. C. COCKERHAM. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

WERLER, J. E., AND J. R. DIXON. 2000. Texas Snakes: Identification, Distribution, and Natural History. University of Texas Press, Austin.

WHELAN, R. J. 1995. The Ecology of Fire. Cambridge University Press, Cambridge, United Kingdom.

WHITLOCK, M. C., AND D. MCCAULEY. 1999. Indirect measures of gene flow and migration: FST≠1(4Nm+1). Heredity 82:117–125.

WITHGOTT, J. H., AND C. J. AMLANER. 1996. *Elaphe obsoleta obsoleta*: response to fire. Herpetological Review 27:145–146.

WOODIN, W. H. 1953. Notes on some reptiles from the Huachuca area of southeastern Arizona. Bulletin of the Chicago Academy of Sciences 9:285–296.

WRIGHT, A. H., AND A. A. WRIGHT, 1957. Handbook of snakes of the United States and Canada. Comstock Publishing Associates, Ithaca, NY.

WRIGHT, B. A. 1941. Habit and habitat studies of the massasauga rattlesnake (*Sistrurus catenatus catenatus* Raf.) in Northeastern Illinois. American Midland Naturalist 25:659–672.

WRIGHT, S. 1978. Variability Within and Among Natural Populations. Vol. 4. University of Chicago Press, Chicago.

YEH, F. C., AND T. J. B. BOYLE. 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129:157.

APPENDIX A

MUSEUM SPECIMENS EXAMINED (*Crotalus willardi*)

ARIZONA: Cochise County, ASU 3787, UAZ 27929, 27930, 27937–27940, 27942, 37757, 39581, Santa Cruz County, UAZ 27931. NEW MEXICO: Hidalgo County, MSB 61241, 61239, 61775, UAZ 40776. CHIHUAHUA: ASU 19927, UAZ 34422. SONORA: UAZ 27936, 27943, 27944, 27945, 28175, 35081.

APPENDIX B

MUSEUM SPECIMENS EXAMINED (*Sistrurus catenatus*)

AMNH 107537; ASU 30148–51, 30153–71, 30577–92, 30621–38, 30877, 30899–906, 32947; MSB 04752, 05232, 17805, 19811, 22091–92, 24362, 30927, 30928, 32059, 32381, 34658, 37967, 41681, 42754, 51881, 51941–42, 52129, 52737, 52891, 52893–94, 53027, 53336, 53913, 54112, 54414, 54904, 55142, 55162–63, 56032, 56286, 56411–12, 56580, 56660, 56663, 56673, 56689–90, 56872, 58680, 59510–11, 60589, 60803–05, 61102, 61228, 61364; MVZ 79231, 209129, 226244; UAZ 45477, 45668; UCM 42373; UNC-MNH 90, 150, 226, 249, 282, 375, 404, 412, 417, 440–42, 450, 494–502, 504–511, 514, 516, 529, 563–64, 568, 575, 601–06, 631, 635, 641, 656, 726, 727, 747, 769, 770, 778–79, 799, 800–01, 816–19, 824–25, 832, 886, 923, 927, 930, 934–35, 938, 968, 971, 977, 983–85, 1004, 1009, 1010, 1044, 1453–54, 1492, 2244; UTA 2902, 2904–05, 9048, 9296, 10996–97, 11279–367, 12676–684, 14081–83, 16393, 19344–45, 19347, 19468, 22372–76, 24518–19, 26538, 28804–09, 30455, 30851–52, 31406, 32386–391, 32397, 32433–35, 32593, 33675, 33777–78, 33955, 34138, 34607, 34703, 34763–64, 34904, 35391, 38806, 38871, 40361–62, 40668, 40817, 40829–839, 40851–52.

APPENDIX C

Sistrurus catenatus catenatus AND *S. c. tergeminus* PREY RECORDS

Summary of original records for prey of *S. c. catenatus* and *S. c. tergeminus*. An asterisk denotes prey consumed in captivity.

Prey	Reference
Arthropoda	
"insect"	Hallock, 1991
"crayfish"	Allen (*pers. comm. in* Reinert, 1978)
Scolopendra spp.	Lardie, 1976
Anura	
Unidentified frog	Ruthven, et al., 1928; Pope, 1926; Netting, 1932; Fox, 1948[a]
Unidentified frog*	Ditmars, 1907; Atkinson and Netting, 1927; Curran, 1935
Acris crepitans	Reiserer, *In press*
Hyla crucifer (*Pseudacris crucifer*)	Atkinson and Netting, 1927; Netting, 1932
Rana spp.	Leray, 1930; Netting 1932; Hallock, 1991
Rana berlandieri	Greene and Oliver, 1965
*Rana clamitans**	Schuett et al., 1984
*Rana pipiens**	Schuett et al., 1984
*Rana sylvatica**	Schuett et al., 1984

Aves	
"bird"	Netting, 1932; Hallock, 1991; This study
"bird"*	Ditmars, 1907
"sparrows"*	Selous, 1900; Loewen, 1947[a]; Best, 1978
"chicks"*	Loewen, 1947[a]
"warbler"	Minton, 1972
Agelaius phoeniceus	Keenlyne and Beer, 1973
Chondestes grammacus (eggs)	Brush and Ferguson, 1986
Colinus virginianus (eggs)	Applegate, 1995
Melospiza melodia	Weatherhead and Prior, 1992
Mammalia	
Unidentified mammal	Taylor, 1892; Greene and Oliver, 1965; Seigel, 1986; Hallock, 1991; This study
"rodent"	Hallock, 1991
"mice"	Taylor, 1892; Netting, 1932; Conant, 1951
"mice"*	Selous, 1900; Ditmars, 1907; Swanson, 1930; Leray, 1930; Wright, 1941; Loewen, 1947[a]; Conant, 1951; Adler, 1960; Keenlyne, 1968; Johnson, 1995
"rabbit"*, "bat"*, "mole"*	Loewen, 1947[a]
"cricetid"	This study

"microtine"	Reinert, 1978; Hallock, 1991
"geomyid"	This study
"soricid"	This study
"zapodid"	Hallock, 1991
Blarina spp.	Johnson, 1995
Blarina brevicauda	Greene and Oliver, 1965; Reinert, 1978; Hallock, 1991; Mauger and Wilson, 1999
Cleithrionomys gapperi	Hallock, 1991
Cryptotis parva	This study
Sorex spp.	Johnson, 1995
Sorex cinereus	Keenlyne and Beer, 1973
Lepus americanus	Weatherhead and Prior, 1992
Microtus spp.	Crawford, 1936; Lyon and Bishop, 1936; Wright, 1941; Mauger and Wilson, 1999; Hallock, 1991
Microtus ochrogaster	Seigel, 1986
Microtus pennsylvanicus	Atkinson and Netting, 1927; Lyon and Bishop, 1936; Conant, 1951; Keenlyne and Beer, 1973; Hallock, 1991
Napaeozapus insignis	Hallock, 1991
Perognathus spp.	This study

Perognathus hispidus	Greene and Oliver, 1965
Perognathus merriami	Greene and Oliver, 1965
Peromyscus spp.	Seigel, 1986; Mauger and Wilson, 1999; Johnson, 1995; Hallock, 1991
Peromyscus leucopus	Wright, 1941; Keenlyne and Beer, 1973; Reinert, 1978
Reithrodontomys montanus	Greene and Oliver, 1965
Zapus hudsonius	Bielema, 1973; Keenlyne and Beer, 1973; Johnson, 1995
Squamata	
Unidentified snake	Ruthven et al., 1928; Conant, 1951; Greene and Oliver, 1965; Keenlyne and Beer, 1973; Hallock, 1991; This study
Heterodon nasicus	Greene and Oliver, 1965
Opheodrys vernalis	Mauger and Wilson, 1999
Sistrurus catenatus	Ruthven, 1911[b]; Hallock, 1991
*Sistrurus catenatus**	Keenlyne 1968
Sonora episcopa (=semiannulata)	Greene and Oliver, 1965
Storeria dekayi	Seigel, 1986; Mauger and Wilson, 1999
*Storeria dekayi**	Johnson, 1995
Storeria occipitomaculata	Reinert, 1978

Thamnophis spp.	Hallock, 1991
*Thamnophis radix**	T. Anton, *pers. comm.*
Thamnophis sirtalis	Keenlyne and Beer, 1973; Seigel, 1986
*Thamnophis sirtalis**	Keenlyne, 1968
Tropidoclonion lineatum	Greene and Oliver, 1965; This study
Cnemidophorus spp.	Klauber, 1972; This study
Cnemidophorus spp.*	Loewen, 1947[a]
Cnemidophorus gularis	Greene and Oliver, 1965
Crotaphytus collaris	Webb, 1970
*Crotaphytus collaris**	Loewen, 1947[a]
Unidentified skink	This study
Lygosoma laterale (*Scincella lateralis*)	Greene and Oliver, 1965;
Phrynosoma cornutum	Greene and Oliver, 1965
Sceloporus olivaceous	Greene and Oliver, 1965

As cited in ([a]) Klauber, 1972 and ([b]) Wright, 1941.

APPENDIX D

LITTER SIZE AND MORPHOMETRICS FROM WILD-CAUGHT PREGNANT *Crotalus willardi*

Date of first exam indicates date maternal SVL was measured and where applicable, date of palpation. Neonatal SVL and mass reported as mean ± *SD* with range in parentheses if available. If the entire litter was not measured, sample size is indicated in brackets. A dash (—) indicates data are not available or not applicable.

Date of 1st exam	Maternal SVL (mm)	Litter size	Date of parturition	Neonatal SVL (mm)	Neonatal mass (g)	Locality	Source
—	~411	2[d]	—	—	—	— (*C. w. silus*)	Klauber, 1949, 1972
—	—	9[e]	—	—	—	— (*C. w. willardi*)	Klauber, 1972
19 Apr	441	6[b]	24 Jul	—	—	Peloncillo Mtns.	C. Painter, pers. comm., MSB 62132–8.

2 May	500	7[b]	9 Aug	169.4 ± 4.3 (165–174) [*N* = 5]	8.3 ± 0.5 (7.5–8.5) [*N* = 5]	San Juanito, Chihuahua	Delgadillo Espinosa et al., 1999
12 May	~505	8[b]	ca. 16 Aug	—	—	Huachuca Mtns.	J. Porter, pers. comm.
17 May	423	6[d]	—	—	—	Animas Mtns.	This study, MSB 61775
17 May	451	6[a]	—	—	—	Animas Mtns.	This study
17 May	488	7[a, b]	3 Aug	168.7 ± 10.1 (150–182)	7.0 ± 0.7 (5.7–7.6) [*N* = 6]	Animas Mtns.	This study
20 May	402	4[a]	—	—	—	Peloncillo Mtns.	This study
22 May	454	5[a]	—	—	—	Animas Mtns.	This study

28 May	450	4[a, b]	ca. 14 Aug	176.3 ± 4.8 (170–180)	7.3 ± 0.5 (7–8)	Huachuca Mtns.	F. Wilson, pers. comm.
2 Jul	502	6[c]	11 Aug	162 [N = 1]	5.75 [N = 1]	Animas Mtns.	This study
3 Jul	~437	6[b]	4 Aug	167.0 ± 5.9 (160–172)	8.3 ± 0.8 (7–9)	Huachuca Mtns.	F. Wilson, pers. comm.
9 Jul	465	4[a]	—	—	—	Animas Mtns.	This study
Jul	—	5[b]	7 Aug	—	—	Sierra de la Purica	Armstrong and Murphy, 1979
Jul	—	4[d]	—	—	—	Sierra de la Purica	Armstrong and Murphy, 1979
15 Jul	~460	6[a, b]	ca. 30 Aug	—	—	Huachuca Mtns.	J. Porter, pers. comm.

15 Jul	438	4[c]	19 Aug	172.0 ± 4.4 (169–177) [N = 3]	7 ± 0.5 (6.5–7.5) [N = 3]	Animas Mtns.	This study
15 Jul	478	4[a]	—	—	—	Animas Mtns.	This study
15–23 Jul	—	4[e]	10 Aug	—	—	Sierra de la Purica	Armstrong and Murphy, 1979
17 Jul	406	5[c]	26 Aug	161.0 ± 1.0 (160–162) [N = 3]	6 ± 0.5 (5.5–6.5) [N = 3]	Animas Mtns.	This study
19 Jul	~420	5[b]	14 Aug	—	—	Santa Rita Mtns.	Quinn, 1977
2 Aug	489	8[a, b]	13 Aug	158.9 ± 2.2 (155–162)	5.3 ± 0.3 (5.0–5.8)	Peloncillo Mtns.	Holycross, 2000

3 Aug	~484	7[b]	18 Aug	—	6.1 ± 0.4 (5.4–6.7) [N = 6]	Santa Rita Mtns.	Martin, 1975b
13 Aug	436	5[d]	—	171.0 ± 1.9 (169–173)	—	Huachuca Mtns.	This study, UAZ 27929

Litter size determined by: ([a]) palpation of embryos in live females, ([b]) a count of all fully-formed neonates in a litter, ([c]) palpation with subsequent observation of a portion of the litter, ([d]) necropsy and ([e]) unknown.

APPENDIX E

VOUCHER SPECIMENS (*Sistrurus catenatus edwardsii*)

ASU: 30577–78, 30581–83, 30585–86, 30592, 30622, 30624–27, 30629–34, 30638, 30877, 30899–906, and 30985.

APPENDIX F

ALLELE FREQUENCIES IN SAMPLES OF *Crotalus willardi obscurus* BY LOCUS

ICC and WFC indicate Indian Creek Canyon and West Fork Canyon demes, respectively.

	San Luis	Peloncillo	Animas (pooled)	Animas (ICC)	Animas (WFC)
***Cw*A14**					
147	0.034		0.046	0.083	0.017
149	0.190				
153	0.052				
155	0.069				
159	0.086		0.157	0.167	0.150
161			0.278	0.167	0.367
163	0.155				
165	0.259	0.667	0.306	0.375	0.250
167	0.155	0.194	0.009	0.021	
169		0.139	0.185	0.188	0.183
175			0.019		0.033

***Cw*A29**					
160	0.034		0.111	0.146	0.083
170	0.466		0.796	0.750	0.833
172	0.138	0.528			
174		0.167	0.074	0.063	0.083
176			0.009	0.021	
178	0.017				
182	0.017				
184	0.121				
186	0.034	0.167			
188		0.083			
190	0.034	0.056	0.009	0.021	
192	0.034				
194	0.052				
196	0.052				

*Cw*B6					
98	0.241	0.056			
118	0.052				
120	0.017				
122	0.310	0.444	0.287	0.354	0.233
124	0.052		0.250	0.292	0.217
126	0.310	0.472	0.361	0.313	0.400
128	0.017		0.065	0.042	0.083
130			0.037		0.067
134		0.028			

***Cw*B23**					
225			0.094	0.104	0.086
227			0.226	0.250	0.207
231			0.009	0.021	
233		0.111			
241			0.009	0.021	
245	0.034		0.066	0.083	0.052
247	0.017				
249	0.207		0.245	0.188	0.293
251	0.155	0.056	0.113	0.167	0.069
253	0.017		0.075	0.063	0.086
255	0.017	0.361	0.066	0.021	0.103
257	0.138				
259		0.111			
261	0.017				
263		0.028			
265	0.017		0.038	0.063	0.017
267	0.034				
269	0.121	0.056	0.019		0.034
271	0.207	0.278	0.038	0.021	0.052
275	0.017				

*Cw*C24					
229	0.017	0.056			
232	0.259	0.056			
235	0.052		0.019	0.022	0.017
238			0.019	0.022	0.017
241			0.019	0.022	0.017
244			0.019	0.022	0.017
247		0.028	0.009	0.022	
250	0.017	0.028	0.047	0.109	
253	0.052	0.111	0.142	0.152	0.133
256	0.052		0.047		0.083
259	0.052		0.047	0.022	0.067
262		0.028	0.019	0.043	
265	0.017	0.139	0.189	0.130	0.233
268	0.017	0.139	0.085	0.065	0.100
271		0.056	0.028	0.043	0.017
274	0.086	0.139	0.047	0.022	0.067
277		0.056	0.028	0.022	0.033
280	0.017	0.083	0.019	0.022	0.017
283			0.009	0.022	
286	0.052	0.028	0.019	0.022	0.017

289			0.009	0.022	
292		0.028			
295	0.069		0.047	0.065	0.033
298	0.034		0.019	0.022	0.017
301	0.086				
304		0.028	0.009	0.022	
310	0.017		0.094	0.087	0.100
313			0.009		0.017
316	0.017				
319	0.086				

***Cw*D15**					
132	0.241				
138	0.138	0.111	0.519	0.583	0.466
141	0.293				
144	0.034	0.222	0.236	0.188	0.276
147	0.121	0.139	0.151	0.125	0.172
153	0.103	0.444	0.047	0.021	0.069
156	0.069	0.083			
159			0.047	0.083	0.017

*Scu*01					
158	0.143				
166	0.018		0.066	0.104	0.034
177	0.357		0.047	0.063	0.034
178	0.268	0.083			
181	0.018		0.198	0.167	0.224
182	0.054	0.639			
183			0.009	0.021	
186	0.089		0.085	0.042	0.121
191	0.036				
194			0.075	0.146	0.017
196			0.057	0.083	0.034
198			0.047		0.086
200			0.283	0.271	0.293
202		0.167	0.009	0.021	
204		0.111	0.123	0.083	0.155
208	0.018				

***Scu*07**					
146	1.000	1.000	0.500	0.478	0.517
148			0.500	0.522	0.483
***Scu*11**					
170	0.315				
172			0.028	0.042	0.017
175	0.056				
176			0.283	0.229	0.328
181	0.037				
182	0.019		0.075	0.063	0.086
184	0.111		0.057	0.104	0.017
194	0.185	0.222			
196	0.037				
198	0.056	0.083			
202	0.074	0.028	0.132	0.146	0.121
204	0.056	0.056	0.113	0.104	0.121
206		0.444	0.066	0.063	0.069
210		0.167	0.104	0.063	0.138
212			0.104	0.104	0.103
214	0.019		0.038	0.083	
216	0.037				